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THE LIFE HISTORY OF *CHOANOTAENIA INFUNDIBULUM*, A CESTODE PARASITIC IN CHICKENS

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INTRODUCTION

Choanotaenia infundibulum, a cestode parasitic in the intestine of chickens, turkeys and several species of game birds, is cosmopolitan in distribution. It is of common occurrence, especially in chickens in many parts of the United States. The house fly, *Musca domestica,* and 2 species of beetles, *Geotrupes sylvaticus* and *Cratacanthus dubius,* have been reported as the intermediate hosts of this tapeworm. Several workers have described parts of the life history of *C. infundibulum,* but the writers have found no records in the literature dealing with the complete demonstration of the life history of this tapeworm. In addition to tracing the entire life cycle of *C. infundibulum,* the present paper reports 9 new intermediate hosts. In 2 of these hosts, the development of the cestode has been demonstrated by infecting chickens as a result of feeding them experimentally infected insects. Gravid segments from the experimentally infected birds were also fed to insect intermediate hosts which became infected, thus demonstrating the complete life cycle under controlled laboratory conditions. A morphological study of the developing cysts has cleared up certain discrepancies in previous descriptions.

REVIEW OF LITERATURE

In a general way the life history of *C. infundibulum* has been known since 1889 when Grassi and Rovelli, in Italy, described from the house fly, *Musca domestica,* cysticercoids which they believed to be those of this species. In Germany, Soloviov (1911) fed a young hen 15 to 20 specimens of *M. domestica* daily during the summer. By late fall the hen was passing segments of *C. infundibulum,* whereas 3 control birds were negative. In the United States, Guberlet (Gutberlet, 1916) fed segments of *C. infundibulum* to 88 flies, *M. domestica,* most of which died within a few days. One out of 6 flies that lived for 12 days contained 5 cysti-

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cercoids of this cestode. In another experiment, Guberlet fed each of 3 chicks 50 *M. domestica* caught around chicken roosts. Three weeks later, post-mortem examinations showed that 1 chick had 6 specimens of *C. infundibulum,* one had 1 specimen of this tapeworm, and the remaining chick as well as 3 controls were negative. Guberlet tried to infect *M. domestica* maggots with *C. infundibulum;* the results were negative. In France, Joyeux (1920) infected a beetle, *Geotrupes sylvaticus,* with *C. infundibulum* but he did not state that he succeeded in infecting chickens by feeding them infested beetles. In the United States, Jones (1930) found a beetle, *Cratacanthus dubius,* containing cysticercoids of *C. infundibulum* but she did not infect a chicken by feeding the infested beetle.

From a review of the literature, it is evident that investigators have obtained both naturally and experimentally infected insects, and that chickens have been infected experimentally with this helminth. However, no previous investigators reported that birds were experimentally infected with cysticercoids as a result of feeding insects experimentally infected with *C. infundibulum.* In other words, the complete demonstration of the life cycle of *C. infundibulum* has not been reported heretofore, so far as the writers have been able to determine from a survey of the literature.

METHODS OF PROCEDURE

Numerous specimens of miscellaneous beetles were collected in chicken yards near Beltsville, Maryland, from 1931 to 1936 (except in one case which is noted elsewhere in this paper). Many of the beetles were dissected to determine whether they harbored the cysticercoids of *C. infundibulum.* If the dissected beetles of a single collection were consistently free from these cysticercoids, the remaining beetles were fed the tapeworm eggs in the following manner. About 12 of the beetles were wrapped in a piece of paper towel containing segments of *C. infundibulum,* and placed on slightly dampened sand in a 50 cc. wide mouthed bottle. This was repeated on 2 or more consecutive days. After having been exposed to infection, the beetles were taken from the bottles on alternate days and the sand was removed and shaken well in a flat pan to dry it. The sand was then dampened and the living beetles wrapped in a piece of paper towel containing mixed mash. The mouths of the bottles were covered with muslin to keep the beetles from escaping. Under these conditions, the beetles lived well provided the sand was kept slightly moist. Beetles were kept for various periods, after offering them segments and before examination for cysts. If the cysts were needed for the study of the larval stages, the beetles were killed at appropriate intervals. Whenever more than 4 apparently infective cysts

of *C. infundibulum* were obtained from a beetle, the cysts were fed to a chicken.

Both nymphs and adult grasshoppers were collected in the open from different locations near Beltsville, Md., during 1935 and 1936. The grasshoppers were kept in screened cages, in groups of from 6 to 12, and fed on mixed mash and lettuce or grass. Individual grasshoppers were force fed, by holding the insect firmly between the fingers, and placing the segments of *C. infundibulum* under the labrum and between the mandibles, then stroking the front and sides of the head until the mandibles started moving. Once the grasshopper chewed and swallowed part of a segment, it usually continued to eat until 3 or 4 segments had been consumed. Only the larger nymphs and adults were fed this way; small nymphs were crushed in the process of handling.

Laboratory-reared grasshoppers for controlled experiments were obtained in the following way. A miscellaneous group of grasshoppers collected around Beltsville, Md., during the fall of 1935, and kept in a large cage, deposited numerous egg masses in dishes of sand. The covered dishes containing eggs that were deposited October 8 to 25, 1935, were first kept in a cool attic, then in a refrigerator at 38° F., later in a laboratory at 60–80° F., and finally after 2 months they were placed in an incubator room at 82° F. Only the eggs of *M. femurrubrum* hatched under the conditions outlined. The nymphs thrived on fresh lettuce and mixed mash.

When the *M. femurrubrum* nymphs were 3 days old and had molted at least once, they were divided into groups of 8 to 12, to prevent cannibalism. All food was withheld from the young grasshoppers for 3 hours before offering them segments of *C. infundibulum* on small pieces of lettuce. The first segments of *C. infundibulum* fed to the nymphs were obtained from fresh fecal samples collected in one infected chicken yard. Segments fed later came from feces which had been collected in the pans under experimentally infected chickens. All infested insects were identified by workers at the U. S. National Museum.

Larval stages of *C. infundibulum* were studied as living and preserved specimens from grasshoppers and beetles. When experimentally infected insects died, they were dissected and the cysts were removed and preserved. Whenever possible, specimens were measured, studied and drawn while still alive. Bouin's picrol formol fixative or 4 per cent formalin was used to fix the cysts. Toto mounts were either cleared for study, or were stained, subsequent to cleaning, with Mayer's alcoholic cochineal, Ehrlich's hematoxylin or Delafield's hematoxylin. It was necessary to dehydrate the cysts and clear them in cedar oil very slowly to prevent undue shrinking. Excellent sections were obtained by staining the cysts in toto in a combination of 1 part Delafield's hematoxylin,

1 part Ehrlich's hematoxylin and 2 parts distilled water, dehydrating, embedding, sectioning 5 to 10 μ thick, washing off the paraffin with xylol and mounting directly in balsam.

Rhode Island Red chickens that had been hatched in the laboratory and kept in tightly screened rooms on clean shavings in the poultry-parasite laboratory at Beltsville, Md., were used as definitive hosts. The method followed in feeding was to partially dissect the insect, in order to be certain that it was infected, preserve 1 to 3 cysts for mounting, and feed the remaining cysts and the insect to a chicken. The chickens were from 6 to 126 days old when fed the cysts of *C. infundibulum*. Until the chickens were 4 weeks old and while they needed hovers, they were kept in tightly screened rooms on shavings, as already mentioned. Chickens more than 4 weeks old were kept in individual compartments in a chicken battery within the screened laboratory building. Some of the birds were killed 10 days after being fed infected material, but the majority were kept for 24 days, or until they passed gravid segments of *C. infundibulum*.

BEHAVIOR OF GRAVID SEGMENTS

The gravid segments of *C. infundibulum* are elongate, funnel-shaped, fragile, white bodies which move freely over the surface of freshly-passed feces. Their characteristic movement and shape have been described by Soloviov (1911). The writers noted that the segments responded in a definite and characteristic manner to the terrain on which the chicken dropped its excrement. When the infected feces fell on grass, the segments ascended the blades, leaving a wavy deposit of extruded eggs behind them. If the grass was only 3 to 4 inches tall, the segments sometimes crawled to the tip of the blades, where they remained actively searching for higher points. Grasshoppers feeding on such plants could easily become infected as a result of ingesting either the segments at the top of the blade, or some of the extruded eggs on the grass. When the infested feces dropped on bare ground, the segments either moved to the edge of the moist area produced by the excrement, or they were found on the damp ground under the feces. Specimens of beetles, *A. granarius,* were frequently found in the damp soil immediately below the chicken excrement; here they could easily ingest some of the eggs. During warm moist weather, the segments were more active than during warm dry weather. Humidity as well as temperature appeared to be an important factor in stimulating the movements of gravid segments of *C. infundibulum*.

EXPERIMENTAL INFECTION OF INSECTS

As already stated, all beetles and grasshoppers used in the following feeding experiments were collected in chicken yards near Beltsville, Md.,

with the following exception. Several specimens of *Aphodius* sp. were collected in a suburban residential district in Chevy Chase, Maryland, a region where no chickens have been kept in recent years. There was very little chance of a natural infestation in these beetles. The experimentally infected specimens, *Aphodius* sp., were definitely not *Aphodius granarius,* which is the common species found in poultry yards around Beltsville.

All of the insects listed in the table were experimentally infected, except *Stenocellus debilipes.* This beetle was examined when brought into the laboratory; at that time cysts of *C. infundibulum* were discovered. It must be kept in mind that there were chances of natural infestations in all of the insects used by the writers because they were collected outside, except in the case of the laboratory-reared grasshoppers. In order to minimize the chances of a natural infestation, the insects were held in the laboratory for 3 to 15 days before being offered tapeworm segments. Part of the insects in each collection were dissected in order to determine if those examined were infested and if the remainder were likely to be infested. Naturally-infested insects are more likely to die during this 3 to 15 day holding period than uninfested insects. If the recovered cysts from a supposedly experimentally-infested insect were in 2 or more very distinct stages of development, it would be logical to assume that the insect might have been naturally infected. Since no cysts varying markedly in the stage of development were recovered from the experimentally infested insects, the probability of a natural infestation could be practically eliminated.

Prior to 1935 all of the chickens that were fed cysts of *C. infundibulum* in the laboratories of the Zoological Division were negative upon post-mortem examination. In some of the cases, this was undoubtedly due to the fact that the cysts fed had not yet developed to the infective stage. Previous to 1935 no records were kept of the ages of the chickens fed and the conditions under which the cysts were fed; both of these factors may have affected unfavorably the retention of the larval cestodes in the final hosts. The writers were not able to infect chickens with infective cysts of *C. infundibulum* which had been kept overnight in a refrigerator.

During 1935 and 1936 a total of 126 beetles, *A. granarius,* were offered segments of *C. infundibulum;* 28 specimens contained from 1 to 100 cysts of this cestode when dissected sometime subsequent to feeding. During the same period, 188 grasshoppers, *M. femurrubrum,* were given segments and eggs on lettuce and 45 of them contained cysts when examined subsequently. The writers' earlier records are not complete for some of these data as indicated in the table, since insects were fed at the infrequent intervals and only when gravid segments were available.

Table of feeding experiments with Choanotaenia infundibulum (*All blank spaces indicate that no data are available*)

Species of insect	Number infected	Date of first feeding	Age of cyst on date of examination (days)	Number of cysts[2] found	Temperatures at which insects were kept (Fahrenheit)	Age of chickens on date of infection (days)	Chickens positive or negative
Beetles							
Stenocellus debilipes[1]	1	....	.....	3	.....	.....	negative
Stenolophus conjunctus	2	Sept. 25, 1931	7	.....	.....	.....	.....
do	2	do	6–13	3, 3+	.....	.....	.....
do	2	do	26	3, 5+	.....	.....	negative
Alphitophagus bifasciatus ..	2	Aug. 20, 1935	24	1, 12	.....	14	negative
do ..	2	do	28	6, 6	.....	.....	.....
do ..	1	May 12, 1936	23	2	.....	.....	.....
do ..	2	June 18, 1936	4– 8	15+, 20	.....	.....	.....
Apocellus sphaericollis	1	Nov. 20, 1935	44–45	1	.....	.....	.....
Ataenius cognatus	1	Aug. 17, 1934	33	.....	.....	.....	negative
do	1	do	44	.....	.....	.....	negative
Aphodius sp.	1	Sept. 15, 1931	5– 6	5	.....	.....	.....
Aphodius granarius	1	Sept. 16, 1931	6	10	.....	.....	.....
do	1	do	8	4	.....	.....	.....
do	1	Oct. 22, 1931	12	7	.....	.....	.....
do	1	April 14, 1933	24	5	.....	.....	.....
do	1	do	31	.....	.....	.....	negative
do	1	March 30, 1936	12	8	.....	.....	.....
do	4	March 31, 1936	49	1, 2, 7, 12	.....	.....	negative
do	1	do	49	4	60–75°	26	positive
do	2	April 7, 1936	50	2, 6	.....	.....	.....
do	1	April 9, 1936	48	4+	60–75°	34	positive
do	1	April 15, 1936	36–37	2	60–75°	.....	.....
do	1	May 26, 1936	8–10	50+	65–75°	.....	.....
do	2	do	13–16	15, 15	65–75°	.....	.....
do	1	do	15–18	1	65–75°	.....	.....
do	1	do	25–27	15	65–75°	32	negative
do	1	do	29–31	4	65–75°	.....	.....
do	1	May 28, 1936	18–19	.....	65–75°	26	negative
do	1	June 1, 1936	15–16	50	65–80°	.....	.....
do	1	do	23–24	4	65–80°	.....	.....
do	1	do	24–25	.....	65–80°	.....	.....
do	2	June 4, 1936	17–18	25, 100+	60–70°	30	negative

Table of feeding experiments—(Continued)

Species of insect	Number infected	Date of first feeding	Age of cyst on date of examination (days)	Number of cysts[2] found	Temperatures at which insects were kept (Fahrenheit)	Age of chickens on date of infection (days)	Chickens positive or negative
Grasshoppers							
Dicromorpha viridis	1	Aug. 16, 1935	20–24	5+	.....	61	negative
Melanoplus femurrubrum ...	1	Sept. 13, 1935	25	1+	.....	35	negative
do ...	1	Oct. 8, 1935	20–23	25	.....	.....	.....
do ...	1	do	21–24	7+	.....	17	negative
do ...	3	do	34–37	.....	.....	24	positive
do ...	1	Oct. 18, 1935	34	.....	.....	30	negative
do ...	1	Jan. 31, 1936	17	4+	75–90°	84	negative
do ...	1	March 6, 1936	12–15	7+	75–90°	.....	.....
do ...	1	do	28–31	10+	75–90°	6	negative
do ...	2	March 10, 1936	34–37	.....	75–90°	11	negative
do ...	1	March 13, 1936	9–10	20+	75–90°	119	negative
do ...	1	do	17–20	.....	75–90°	126	positive
do ...	1	do	29–32	30+	75–90°	12	positive
do ...	2	do	36–39	.....	75–90°	19, 19	positive
do ...	2	do	36–39	.....	75–90°	56, 56	positive
do ...	1	March 16, 1936	36	.....	75–90°	19	positive
do ...	1	do	36	.....	75–90°	56	positive
do ...	1	do	28	.....	40–65°	.....	.....
do ...	1	do	42	2	40–65°	.....	.....
do ...	1	March 25, 1936	21	.....	75–90°	13	negative
do ...	1	do	23	.....	75–90°	15	positive
do ...	2	March 26, 1936	22	.....	75–90°	58	positive
do ...	2	do	22	.....	75–90°	16	positive
do ...	1	March 27, 1936	31	50+	40–65°	.....	.....
do ...	1	do	45	.....	40–65°	.....	.....
do ...	1	do	31	.....	40–65°	.....	.....
do ...	1	July 23, 1936	15	.....	.....	.....	.....
do ...	1	Aug. 6, 1936	14–15	.....	65–80°	51	negative
do ...	1	do	8	.....	75–90°	.....	.....
do ...	1	do	9	.....	65–80°	.....	.....
do ...	1	do	16	.....	65–80°	51	negative

[1] The naturally infested *Stenocellus debilipes* was examined Aug. 21, 1931.
[2] A plus (+) indicates that the insect was not completely dissected so that there may have been more cysts than were counted.

EFFECTS OF TEMPERATURE ON DEVELOPMENT OF CYSTS

Since little is known concerning the rate of development of cestode larvae in invertebrates kept at different temperatures, the following experiments were undertaken. Three day old, laboratory reared *M. femurrubrum* were divided into 2 groups. Group 1 was kept in a warm laboratory where the temperature ranged from 75° to 90° F., thus approximating summer temperatures. Group 2 was kept in an unheated room where the temperature ranged from 40 to 65° F., approximating early spring and fall temperatures. The nymphs in group 1 ate greedily of the segments and lettuce offered, but those in group 2 did not eat. It was found necessary to bring the nymphs of group 2 into the warm laboratory for a few days, feed them the gravid segments on lettuce, and then return them to the unheated room. The approximate temperatures at which other infected grasshoppers and beetles were kept were also noted for comparison.

In the grasshoppers of group 1, the cysts of *C. infundibulum* were infective in 17 to 20 days. In those of group 2, the cysts were still in the "solid ball stage" 28 days after feeding. During the 45 days that the grasshoppers of group 2 were in the unheated room, the cysticercoids developed very little, and the nymph hosts did not appear to grow or molt. Further work on group 2 was discontinued, because many of the nymphs died. When the surviving nymphs of group 2 that had been in the cold room 31 days were placed in the warm room with group 1, the former grew and molted rapidly. After these members of group 2 had been in the warm room for 30 days, the cysticercoids dissected from one nymph appeared to be in the infective stage. The 31-day period in the cold room, during which time the cysticercoids developed very slowly, apparently did not hinder the later development of the larval worms under more favorable conditions.

Larvae in which the head, neck and bladder were differentiated were obtained from grasshoppers fed 8 days previously and kept at 75 to 90° F.; from grasshoppers fed 14 to 15 days previously and kept at 65 to 80° F.; and from beetles fed 17 to 18 days previously and kept at 60 to 70° F. Cysticercoids in which the head had envaginated into the bladder were obtained from grasshoppers fed 9 to 10 days previously and kept at 75 to 90° F.; and from beetles fed 15 to 16 days earlier and kept at 65° to 80° F.

TOTAL LIST OF INTERMEDIATE HOSTS IN WHICH *C. infundibulum* CYSTS OCCUR

The following species have been reported as intermediate hosts for *C. infundibulum:* House fly, *M. domestica;* beetles, *Geotrupes sylvaticus, Cratacanthus dubius, Stenocellus debilipes,* Stenolophus conjunctus,**

*Alphitohagus bifasciatus,** *Apocellus sphaericollis,** *Ataenius cognatus,** *Aphodius* sp.* and *Aphodius granarius;** grasshoppers, *Dicromorpha viridis** and *Melanoplus femurrubrum.**

MORPHOLOGY OF LARVAE

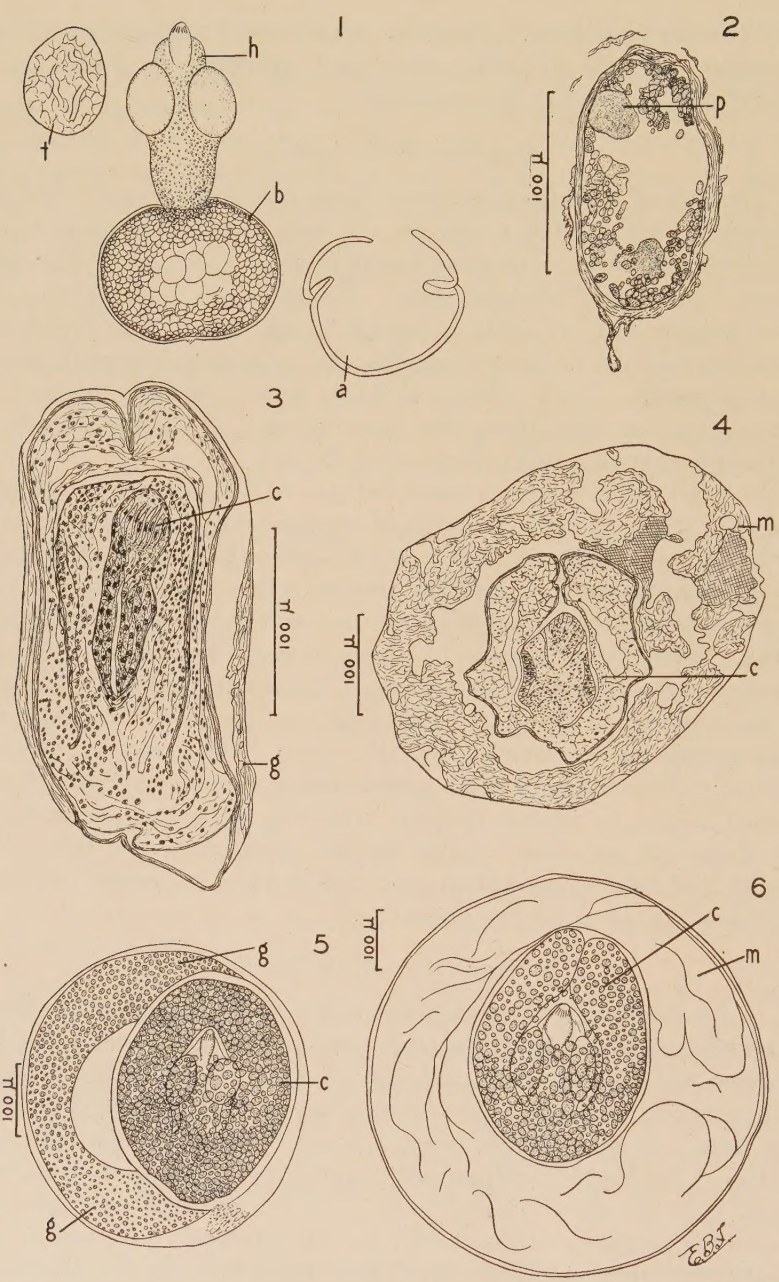
The eggs of *C. infundibulum* are about 65 μ long by 40 μ wide, the oncospheres are about 32 μ long by 23 μ wide and the embryonal hooks are about 18 μ long. The cysts found in beetles fed 5 to 6 days previously were in the "solid ball stage." Examinations of sectioned specimens indicate that no cavity is present in the larva. This "solid ball stage" varied from 117 to 300 μ in diameter in living specimens subjected to no pressure. The 6 embryonal hooks were sometimes observed in these larvae; at other times they were absent. A distinct capsule or external membrane 5 to 8 μ thick covers the ball.

In larval cestodes from a beetle fed 8 to 10 days previously and kept at 65 to 75° F., an irregular cavity of varying proportions has begun to form. It appeared that the cavity in the "hollow ball stage" was formed by the disappearance of centrally located cells. Examination of sectioned material showed that there was no membrane which bounded this cavity. Frequently, strands of tissue crossed the cavity. Several rounded protuberances of homogeneous tissue extended from the wall of the larva toward the cavity (plate 1, fig. 2). As the opening enlarged, these protuberances extended into the cavity. There was no apparent internal differentiation of these protuberances, and the embryonal hooks seemed to bear no relation to them. In living specimens, the larva did not always fill the entire capsule but left space along one side. The "hollow ball stage" varied from about 160 to 350 μ in diameter in living specimens not subjected to pressure.

DIFFERENTIATION OF LARVAE

In the next stage of development observed, the larva had elongated and 3 sections of the body, the head, neck and bladder, could be readily distinguished. The rostellar sack and rostellar hooks were beginning to form, and masses of cells indicated the future location of the 4 suckers. The neck was the undifferentiated area which connected the head and bladder. The bladder, which usually contained the embryonal hooks, was a solid mass of cells of varying sizes. The central tissue was loose, and toward the periphery of the bladder the cells gradually decreased in size towards the outermost layers which were dense and slightly pigmented. The cuticula of the bladder was well developed, in contrast to that covering the head and neck.

* The species designated with an asterisk are reported as intermediate hosts for the first time.



EXPLANATION OF FIGURES

All drawings except figure 1 were made with the aid of a camera lucida.

a, cyst wall; b, bladder; c, cysticercoid; g, granular tissue in cyst; h, head; m, mucoid tissue in cyst; p, protuberance; and t, tail.

The living cysts, not under pressure, in which parts of the larvae were beginning to differentiate varied from about 200 to 500 μ in diameter. Larval cestodes from a single intermediate host that had been fed segments only once varied in development as well as in size; it appears that the heavier infestations cause greater variation in size, some of the cysticercoids developing more slowly than others.

During the stage of development just described in living specimens, a tail could be found which was located within the external membrane. Living grasshoppers were cut into pieces and the cysts promptly removed. The external membrane of the cyst was immediately slit, and its contents gently forced out into tap water. The larva exuded first, followed by a colorless granular mass, the tail, which was surrounded by a very fragile covering (plate 1, fig. 1). This membrane was easily broken and soon ruptured in tap water or physiological salt solution. In all the material observed, the tail seemed to be a solid but loose mass of cells which was held together by the surrounding membrane. No connection between the tail and bladder could be demonstrated in living material, but there were indications of a few loose strands of tissue which might have been connections. Sections of a cyst in a little later stage of development showed a distinct but loose connection between the bladder and what seemed to be the remains of the tail. When the larva and tail were forced out of the living cyst, the external membrane was left clean and without any lining.

The rostellar hooks and suckers were almost completely differentiated before the invagination of the head. The larva lying within the external membrane elongated so that the head was folded back against the bladder. After invagination the first definite movements of the head were visible. When the external membrane was pricked, the contents popped out as though held under considerable pressure.

The head of the larva developed rapidly until 4 weakly muscular but distinct suckers and 16 to 24 rostellar hooks (17 to 22 μ long) were visible. At this stage, the larvae appeared to be completely formed and contained only a few calcareous corpuscles. The larvae easily evaginated from the bladder when placed in tap water. The writers were not able to infect the primary hosts with such cysts, obtained either from beetles or grasshoppers.

Fig. 1. *Choanotaenia infundibulum;* cysticercoid and tail dissected from living cyst obtained from *M. femurrubrum.*

Fig. 2. *C. infundibulum*; section 5 μ thick, of cyst in "hollow ball stage," from *A. granarius.*

Fig. 3. *C. infundibulum;* section of infective cyst, 6 μ thick, from *A. granarius.*

Fig. 4. *C. infundibulum,* section of infective cyst, 5 μ thick, from *M. femurrubrum.*

Fig. 5. *C. infundibulum,* toto mount; cyst from *A. granarius.*

Fig. 6. *C. infundibulum,* toto mount; cyst from *M. femurrubrum.*

INFECTIVE CYSTS

Due to changes in the tail tissue in later development, the cysts of *C. infundibulum* from beetles and from grasshoppers were very different in appearance and size as shown in plate 1, figs. 5 and 6. The living invaginated larvae from both intermediate hosts were about 350 by 250 μ when not subjected to pressure. The internal structure of the larvae from the 2 hosts was identical in all respects. The scolex lay within the invagination cavity, entirely surrounded by the bladder. Numerous calcareous corpuscles in the head and bladder masked the internal structure. The entire cyst had a yellowish color that seemed to be correlated with infectivity. From 17 to 20 days at a temperature of 75 to 90° F. is necessary for the development of cysticercoids to the infective stage in *M. femurrubrum.* The writers were able to infect chickens with cysticercoids that were 48 days old or older, the cysticercoids being obtained from *A. granarius* that had been kept at a temperature of 60 to 75° F. It is probable that a shorter period of development at higher temperatures, as used in the grasshopper feeding experiment, would also produce infective cysticercoids of *C. infundibulum.*

The live cysts removed from beetles ranged from about 350 to 450 μ in diameter. The cysts were more or less spherical in shape. Many cysts without any indication of an external membrane or tail tissue were found in beetles. At the other extreme, cysts containing the cysticercoid and many loose cells were found in the same intermediate host (plate 1, fig. 3). The great difference in the size of these cysts was due mainly to the variation in the quantity of loose cells, probably degenerating tail tissue. These loose cells were scattered around the cysticercoid and tended to collect as a lining for the external membrane. When the external covering of a living cyst was slit, these cells came out as loose masses without any definite arrangement, leaving a clean external membrane. The fact that these cells were not connected with the external capsule indicates that they were probably a remnant of similar tail tissue observed earlier in development. A study of sectioned specimens confirmed the presence of the loose cells seen in the living cysts. Sectioned cysts treated with Ehrlich's and Delafield's hematoxylin stains showed a distinct difference in the color response of the cysticercoids and the loose cells. The nuclei of the cysticercoid cells took a bright blue stain, in contrast to the dirty brown grey color of the loose cells.

Infective living cysts from the grasshopper were about 500 to 800 μ in diameter. More than half the volume of the cyst was occupied by a very convoluted mucous-like brown mass without any cellular structure. The larva usually lay near one side of the cyst, and the mass surrounded the larva and occupied the remainder of the cyst, (plate 1, fig. 4). When a living cyst was slit open, the mucoid mass could be forced out of the

cyst as a unit, and it did not break up in water readily. Several parallel-walled channels extended through this mass, and sections of an infective cyst confirmed the presence of these channels. No loose cells similar to those observed in the cysts from beetles were seen in either living or preserved cysts from the grasshopper. The mucoid mass was present in all the cysts from the grasshopper; but the quantity of it varied. Unlike the 2 types of cysts that were obtained from beetles, only this one type of cyst was obtained from the grasshoppers.

The infective cysts from both grasshoppers and beetles have 2 common characteristics of importance. These are as follows: (1) With but one exception, numerous calcareous corpuscles were present in all the cysticercoids which the writers demonstrated to be infective; (2) The external cyst wall of the infective stage had a distinctly yellow color in contrast to the grey color of the non-infective cyst. Structurally, infective cysts of *C. infundibulum* may be characterized as simple cysticercoids with heads similar to those of the adult, without a tail and with numerous calcareous corpuscles.

DISCUSSION

Grassi and Rovelli, Guberlet and Joyeux have described some larval stages of *C. infundibulum* found in house flies and beetles, but the early development of the cysts has not been reported heretofore. Grassi and Rovelli's (1889, 1892) description of the cyst is so brief, and their figure is so diagrammatic, that it is impossible to estimate the age of the larvae studied by these writers, except that the cysts were well-developed. Guberlet (1916) described 12-day old cysts from the house fly, *M. domestica,* which were well developed but probably not infective. Joyeux (1926) described 17 to 26-day old cysts from the beetle, *Geotrupes sylvaticus*. The older cysts may have been infective. None of these workers reported infecting birds with experimentally infested insects.

Grassi and Rovelli observing cysticercoids from house flies, and Joyeux using larval stages from beetles, reported no tail in the cyst. Guberlet described and figured a tail (bladder in his terminology) in the cyst from the house fly. Through the courtesy of Dr. Guberlet and Dr. Henry B. Ward, some of Guberlet's sectioned material was borrowed from Dr. Ward's collection. Examination of these sections from the house fly, which contained several 12-day old cysts embedded in tissue, showed a very definite tail similar to that illustrated in figure 12, plate 6 of Guberlet's paper (Gutberlet, 1916). In all of Guberlet's specimens that were examined by the writers, the head was invaginated. In all of the writer's specimens in which a tail was observed, the head had not invaginated. This indicated that Guberlet's specimens had developed further than those of the writers' in which a tail was present. It is evident from Guberlet's and the writers' observations that a tail is present

during one period of the development of the cyst. None of the writers' sectioned specimens show a hollow tail as do those of Guberlet. In one of Guberlet's sectioned specimens, as he illustrated, the embryonal hooks lay within the tail, but in all the other specimens examined the hooks were on or in the bladder.

Joyeux described 26-day old cysts from beetles similar to the infective ones found by the writers, but that author did not interpret the loose cells lying outside the cysticercoid in the same way as the writers do. The cysts studied by Joyeux were about 330 μ in diameter, had no tail and were surrounded by an adventitious membrane with a structure that was difficult to see. Joyeux believed that the adventitious membrane consisted of cuticula and an inner region of varying thickness. It seems probable that this inner region was the same as the loose cells which the writers believe originated as tail tissue. Several cysticercoids without external membrane and loose cells were obtained from the writers' dissected beetles. This sctioned material, a completely developed cysticercoid embedded in beetle tissue, had neither external membrane nor loose cells. Since the sectioned tissue was not broken, this cysticrcoid probably lost the external membrane and loose cells during development. The lack of these two structures indicates that cysticercoids without covering may be found in beetles normally. One chicken was fed 6 cysticercoids from a beetle with negative results; the larvae were without an external membrane and loose cells.

EXPERIMENTAL INFECTIONS OF CHICKENS

Chickens were infected with *C. infundibulum* by feeding them cysts from the beetle, *A. granarius,* and the grasshopper, *M. femurrubrum.* The table gives the results of the feeding experiments. Control birds kept with the experimental birds were negative upon post-mortem examination.

It was found by daily examination of feces, that birds passed gravid segments of *C. infundibulum* 13 to 15 days after being fed the cysts. Only those chickens that were more than 12 days old when fed became infected with this cestode. One chicken that was fed 1 laboratory raised, experimentally infected *M. femururbrum* contained 39 adult *C. infundibulum* upon post-mortem examination; this was the maximum number obtained from any of the writers' experimentally infected chickens. A total of 22 chickens were fed what were probably infective cysts of *C. infundibulum* from *M. femurrubrum* and 12 of these birds were positive upon post-mortem examination. Out of 7 birds that were fed what were probably infective cysts from *A. granarius* only 2 contained this cestode.

CROSS INFECTION OF INSECTS

In order to prove that the writers were dealing with only 1 species of cestode, cross infection experiments were undertaken with different insects. Three laboratory raised *M. femurrubrum* nymphs were fed freshly passed segments of *C. infundibulum* from an experimentally infected chicken. Seventeen to 20 days later the grasshopper contained cysts which were fed to a laboratory raised chicken. The gravid segments from this chicken were offered to 24 specimens of *A. granarius,* collected under conditions described previously. Twenty-five to 27 days later 1 of these beetles contained cysts of *C. infundibulum;* the cysts were fed to 2 laboratory raised chickens. These chickens were examined post mortem 15 days after feeding but were negative for *C. infundibulum.* In spite of the fact that the infective cysts described from the beetle and grasshopper are different in appearance, there is no doubt that they are both larvae of *C. infundibulum.*

DEFINITIVE HOSTS OF *C. infundibulum*

C. infundibulum has been reported from the following birds: Domestic chicken, *Gallus gallus;* turkey, *Meleagris gallapavo;* Barbary partridge, *Alectoris barbara* (syn. *Caccabis petrosa*); red-legged partridge, *Caccabis rufa;* European quail, *Coturnix coturnix;* European partridge, *Perdix perdix;* "European" pheasant, *Phasianus colchicus;* Chinese francolin, *Francolinus pintdeanus;* Indian black kite, *Milvus govinda;* and little bustard, *Otis tetrax.*

SUMMARY

1. The following species of new intermediate hosts for *C. infundibulum* are reported: *Stenocellus debilipes, Stenolophus conjunctus, Alphitophagus bifasciatus, Apocellus sphaericollis, Ataenius cognatus, Aphodius* sp., *Aphodius granarius, Dicromorpha viridis* and *Melanoplus femurrubrum.*

2. Laboratory-raised chickens have been infected with *C. infundibulum* by feeding them cysts from experimentally infected *A. granarius* and *M. femurrubrum.*

3. A tail has been demonstrated in partially developed cysts from *M. femurrubrum.*

4. At 75 to 90° F., 17 to 20 days is the minimum time for development of the cysticercoids to the infective stage in *M. femurrubrum.* In the beetle, *A. granarius,* 48 days is the minimum time for development at 60 to 75° F.

5. Infective cysts are simple cysticercoids without a tail, with or without an external membrane and loose cells or mucoid tissue, and with numerous calcareous corpuscles.

6. Cross infestion experiments prove that the beetle and grasshopper cysts are the same species in spite of apparent differences.

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SPECIES AND INCIDENCE OF DIPTEROUS LARVAE CONCERNED IN WOUND MYIASIS

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It is known that a number of species of Diptera, principally in the family Calliphoridae, and to a lesser extent in the family Sarcophagidae and other families, are concerned in the production of myiasis in domesticated animals. Information available in the literature as to the species implicated and the comparative incidence of each is limited. Since the discovery, by Cushing and Patton (2), that the screwworm, *Cochliomyia americana* C. and P., has been confused with *Cochliomyia macellaria* Fab., there has been a greater interest in determining the importance of the various species of myiasis-producing Diptera.

Bishopp (1) discusses several species of Calliphoridae which cause myiasis in man and animals, namely, *C. macellaria* Fab., *Phormia regina* Meig., *Lucilia sericata* Meig., *L. illustris* Meig.,[2] *Calliphora erythrocephala* Meig., and *Cynomyia cadaverina* Desv. Parish and Laake (4) record the species of flies concerned in the production of myiasis in Menard County, Texas, as based on the rearing of 46,242 adults. The number of infested animals from which these adults were reared is not mentioned. From these flies, which were reared at different times over a period of three years, it was found that *Cochliomyia* spp. (no differentiation was made between the two species, *i.e., C. americana* and *C. macellaria*) constituted approximately 94 per cent of all flies reared, whereas approximately 6 per cent of the flies were *Phormia regina.* Two specimens of *Sarcophaga plinthopyga* Wied. and two of *Sarcophaga* sp. were also reared. Laake, Cushing, and Parish (3) record the number of species of flies reared from 114 cases of myiasis in all classes of range livestock on one ranch near Sonora, Tex. An analysis of these shows 79.8 per cent of the cases involved the primary screwworm, *C. americana,* in pure culture, 10.5 per cent were mixed cultures of *C. americana* and secondary species,[3] such as *C. macellaria, P. regina, L. sericata,* and *S. plinthopyga,* whereas 9.6 per cent were pure cultures of secondary species involving the same species as those mentioned. The primary

[1] The authors are indebted to all those cooperating in obtaining specimens from wounds of animals and especially to their associates in the Bureau of Entomology and Plant Quarantine by whom most of the specimens were collected.

[2] Generally identified as *Lucilia caesar* L. heretofore. *L. caesar* is not known to occur in the United States.

[3] The term "secondary species" is applied to the wound-invading species, other than *C. americana,* which, although ordinarily necrobiotic, may cause wound myiasis in man and animals.

screwworm fly was involved in 90.4 per cent of the total number of cases, while secondary species were implicated in 20.2 per cent of the total.

In the present paper the authors present results of determinations of 901 lots of larvae collected from wounds of man and animals in the southeastern, middle western, and southwestern sections of the United States. The majority of the larvae, however, were collected in the States of Georgia, Florida, South Carolina, Alabama, and Louisiana. The data herein reported would therefore probably be more applicable to conditions existing in those States.

TABLE 1.—*Myiasis-producing larvae collected from animals in the various States or areas during 1935 to April 1, 1936*

State or area	Number of lots of larvae	Number of specimens of larvae	Number of cases of *Cochliomyia americana*	Number of cases of secondary species	Number of mixed infestations of *C. americana* and secondary species	Percentage of cases involving *C. americana*	Percentage of cases involving secondary species
Florida	224	4,572	215	4	5	98.2	4.0
Georgia	162	3,122	135	18	9	88.9	16.7
South Carolina	157	2,460	144	11	2	93.0	8.3
Louisiana	149	1,824	128	17	4	88.6	14.1
Alabama	91	1,071	66	24	1	73.6	27.5
Mississippi	21	150	3	18	0	...	...
Illinois *et al.*[1]	67	774	58	7	2	89.6	13.4
Texas *et al.*[2]	22	377	19	3	0	86.4	13.6
State unknown	8	50	1	7	0	...	...
All areas combined[3].	880	14,259	766	91	23	89.7	12.9

[1] Includes Illinois, Tennessee, Missouri, Oklahoma, Arkansas, and Kansas.
[2] Includes Texas, New Mexico, Arizona, and California.
[3] Since Mississippi was largely free of *C. americana* during the time specimens were collected, the data from this State are not included in calculating the comparative incidence of *C. americana* and secondary species.

TABLE 2.—*Secondary wound-invading flies and incidence of each*

Species or genus	Cases in which only secondary species were involved	Cases with mixed infestations of *C. americana* and secondary species	Total incidence of each genus or species
	Number	*Number*	
Phormia regina Meig.	63	16	79
Lucilia spp.[1]	34	4[2]	38
Cochliomyia macellaria Fab.	12	4	16
Sarcophaga spp.	3	5	8[3]
Calliphora spp.	3	0	3
Musca domestica L.	2	0	2
Cynomyia cadaverina Desv.	1	0	1
Stomoxys calcitrans L.	1	0	1
Hermetia spp.	1	0	1
Undetermined	2	0	2

[1] Species of *Lucilia* and total incidence of each are as follows: *L. sericata* Meig., 18; *L. caeruleiviridis* Macq., 10; *L. cluvia* Walk., 3; and *Lucilia* (undetermined) 7.
[2] All *L. sericata* Meig.
[3] *Sarcophaga bullata* Park. implicated in 5 of the 8 cases.

The larvae were collected from wounds of the various hosts by the personnel of the Division of Insects Affecting Man and Animals, Bureau of Entomology and Plant Quarantine, and through other sources during the period June, 1935, to April 1, 1936. The majority of the cases, however, were collected during the months of July through November, 1935.

The results of the determinations are given in condensed form in tables 1, 2, and 3.

The information contained herein, it is believed, serves as a fair index to the incidence and importance of the primary myiasis-producing species, *Cochliomyia americana,* as compared with other species of wound-invading flies.

The incidence as recorded from the data emphasizes the importance of *Cochliomyia americana* as a myiasis-producing species. *C. americana,* using the cases herein reported as a criterion, was implicated in approximately 90 per cent of all cases of myiasis in regions where *C. americana* was present. It is known that *C. americana* larvae in general are more harmful to the host than are the larvae of the secondary species; therefore the importance from the standpoint of actual damage would, no doubt, be considerably greater than the incidence indicates.

The collections indicate, however, that secondary forms should be given some consideration, since secondary invaders were involved in approximately 13 per cent of all the cases (table 1).

The importance of the secondary species from the standpoint of degree of injury to the host is not well known. No doubt in some cases the larvae are beneficial to the wound, but in others, where larvae are present in the wounds in large numbers, it is known that they do considerable harm to the host and may even cause death.

The species of secondary flies involved in myiasis and the incidence of each are shown in table 2. Since in a number of cases several species were present in the wound at the same time, the total number of infestations is not so great as the total incidence of all species. *Phormia regina* was the most common secondary form and from these collections appears to be the most important of the secondary species. *Lucilia* spp. as a group ranked second in the number of cases in which the various species were involved. The species of this genus taken from wounds were *L. sericata, L. caerulei viridis* Macq., *L. cluvia* Walk., and possibly others. *L. caeruleiviridis* and *L. cluvia* apparently have not previously been reported as causing myiasis in animals. *Cochliomyia macellaria* ranked third in the number of cases in which the species was taken. *Sarcophaga* spp. as a group was fourth. Out of eight infestations involving species of this genus, *S. bullata* Park. was taken in five cases. Other genera or species taken were *Calliphora* spp., *Musca domestica* L., *Cynomyia cadaverina, Stomoxys calcitrans* L., *Hermetia* spp., and two undetermined species. The infestations involving *M. domestica* and

TABLE 3.—*Number of cases involving* Cochliomyia americana *and secondary wound-invading species in the various hosts in each State or group of States*

Host	Florida		Georgia		South Carolina		Louisiana		Alabama		Illinois *et al.*[1]		Texas *et al.*[2]		Mississippi		State unknown		All areas combined	
	Cochliomyia americana	Secondary species	*C. americana*	Secondary species	*C. americana*	Secondary species	*C. americana*	Secondary species	*C. americana*	Secondary species	*C. americana*	Secondary species	*C. americana*	Secondary species	*C. americana*	Secondary species	*C. americana*	Secondary species	*C. americana*	Secondary species
Man	4	1	1	3	0	0	1	0	0	1	0	1	1	0	0	2	0	1	7	9
Cattle	113	2	46	7	64	6	39	9	34	13	24	6	13	2	1	5	1	1	335	51
Swine	72	2	51	8	52	5	36	3	20	6	22	2	2	0	1	2	0	0	256	28
Horses	13	1	9	0	4	0	12	1	4	1	6	0	2	0	1	0	0	0	51	3
Sheep	0	1	4	2	2	0	38	5	0	1	3	0	0	1	0	0	0	0	47	10
Mules	8	0	15	1	16	1	1	0	3	1	2	1	0	0	0	1	0	0	45	5
Dogs	2	0	9	0	5	1	1	0	0	0	2	0	0	0	0	1	0	0	19	2
Goats	7	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	9	1
Cats	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	2	2
Antelope	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0
Turkeys	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1
Chickens	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Geese	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
Ducks	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Opossum	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Gopher tortoise[3]	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Unrecorded	0	0	7	2	3	0	3	2	5	2	0	0	0	0	0	4	0	5	18	15

[1] Includes Illinois, Tennessee, Missouri, Oklahoma, Arkansas, and Kansas.
[2] Includes Texas, New Mexico, Arizona, and California.
[3] *Testudo polyphemus* Daudin.

especially *S. calcitrans* were probably accidental, although *M. domestica* has been bred from old necrotic wounds on experimental animals.

The numbers of cases of myiasis, by States as well as for the entire area which were caused by *Cochliomyia americana* and secondary species in the various classes of animals are presented in table 3. Although many factors enter, such as population of various classes of animals, ease of collecting larvae from the different types of animals, etc., which may make this information somewhat unreliable, these data indicate that cattle and swine were the principal hosts for *C. americana* and also for the secondary species.

Although the number of cases involving secondary species represented only about 13 per cent of the total cases, this group of flies was taken from a greater variety of host animals than was *Cochliomyia americana.*

SUMMARY

Cochliomyia americana, the primary screwworm fly, was involved in approximately 90 per cent of the total number of cases of myiasis, whereas secondary species as a group were involved in approximately 13 per cent of the total number of cases.

The following species or genera, given in order of their incidence, were taken from wounds: *Cochliomyia americana, Phormia regina, Lucilia* spp., *C. macellaria, Sarcophaga* spp., *Calliphora* spp., *Musca domestica, Cynomyia cadaverina, Stomoxys calcitrans,* and *Hermetia* spp.

Of the species in the genus *Lucilia, L. sericata* was the most commonly taken in myiasis. *L. caeruleiviridis* and *L. cluvia* were also concerned in the production of myiasis. Apparently the two last mentioned species have not previously been reported from wounds of animals.

Sarcophaga bullata is the most common species of the Sarcophagidae concerned in myiasis in the Southeast.

Secondary species as a group apparently attack a greater variety of hosts than does *Cochliomyia americana.*

A host list for *Cochliomyia americana* and the secondary species is given.

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TWO NEW SCHISTOSOMID TREMATODES FROM WATER-BIRDS

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Haemophilous forms have long been considered among the most pathogenic helminths of warm-blooded vertebrates and of these the schistosomid trematodes have occupied a prominent place.

During the opening years of the present century extensive investigations of those forms infesting human beings resulted in the knowledge of the first complete life-history of a schistosomid trematode. Following this the species, most important from a medical and veterinary standpoint, were fully investigated. Since that time the life-history studies of a number of species have been completed. Periodically from various parts of the world new species of both adults and larvae have been recorded but in many cases the relationship between the two stages is unknown or unrecognized. Interest in many of these has been somewhat passive because, as parasites of wild animals, they lacked great medical or economic importance.

The past decade has witnessed a renewal of interest in these organisms as a whole, partly because a number of larval species are responsible for the production of a transitory dermatitis of man, and partly because of the present realization of the necessity for the conservation of wild life.

In Western Canada the larval stages of the organisms which bear the potentiality for dermatitis production are widespread and the necessity of developing an effective method for their control is urgent. Prairie lakes and sloughs in most cases abound with molluscs capable of acting as intermediate hosts to the Trematoda. Large numbers of water-birds are found in the vicinity of these shallow waters either as migrants or summer residents and, in the work here to be reported, theoretically constitute ideal definitive hosts to the schistosomes. The coincidence of molluscs, birds, and cercariae has been carefully checked over a considerable area in Manitoba and the findings lend support to this theory.

The collection of a representative sample of each of so many bird species is difficult. Also in the case of most schistosomid parasites of birds, the possibility of larvae gaining entrance to the host is so great that high fecundity in the adult is not necessary to insure the survival of the species. As a result, eggs for experimental work are relatively scarce and difficult to recover. This, coupled with the high mortality of

some experimental wild birds in captivity, retards progress to a marked degree.

The following specimens were examined for schistosome parasites during the past year.

Name	*Common Name*	*Number Examined*	*Number Infested*
Larus franklini	Franklin's gull	3	—
Larus argentatus	Herring gull	8	3
Dafila acuta	Pintail duck	12	—
Querquedula discors	Blue-winged teal	30	18
Anas platyrhynchos	Mallard duck	7	—
Nyroca valisneria	Canvasback duck	2	—
Spatula clypeata	Spoonbill duck	11	—
Mareca americana	Baldpate duck	1	—
Marila marila	Bluebill duck	2	—
Nyroca americana	Redhead duck	2	—
Fulica americana	Coot	6	—
Erismatura jamaicensis	Ruddy duck	1	—
Colymbus nigricollis	Eared grebe	2	—
Limosa fedoa	Marbled godwit	1	—
Phalacrocorax auritus	Crested cormorant	1	—
Bartramia longicauda	Upland plover	1	—
Botaurus lentignosus	American bittern	1	—

Total number of species—17. Total number of specimens—91.

The eight herring gulls listed above were collected in the vicinity of Yarmouth, N. S. Canada, and the viscerae were very generously passed on to the writer for examination by Prof. R. A. Wardle. The remaining specimens listed were obtained from hunters or collected personally. A few were picked up dead in marshes but only those in a favorable state of preservation to allow for an adequate examination are included. Specimens of *Querquedula discors* were collected from scattered lakes and sloughs over an area of about two thousand square miles, and the 60% incidence of infestation was fairly constant.

PROCEDURE

Material was examined in as fresh a condition as possible, preferably a few minutes after collecting, because the minute forms such as *Pseudobilharziella sp.* are very difficult to recover after blood coagulation has begun. In spring and early summer the coagulation time of the blood of water-birds in the vessels is sufficiently long to allow for a leisurely examination within one or two hours. In late summer and fall, however, this coagulation time appears to be greatly reduced, and to be of maximum value examinations must be carried out within a few minutes of the time of death. Otherwise the worms may be overlooked or be so badly entangled in blood coagula as to be of little value.

The common procedure was to remove the entire ventral wall of the body cavity and examine the blood vessels *in situ*. During the examination the viscera was sprayed at short intervals with a warm solution of sodium oxalate. This prevented coagulation of the blood when the vessels were opened and also desiccation or lowering of temperature sufficient to kill the parasites. Bright light is necessary or the specimens will be overlooked entirely. In the case of immature worms it was found advantageous to remove the mesentery with its vessels and also the liver to a bath of warm sodium oxalate solution where they could be recovered with the aid of a binocular. Specimens were transferred to a dish of warm physiological saline in which they relaxed and uncoiled somewhat and were then fixed in hot corrosive-acetic solution.

Alum-cochineal was found to be the superior stain for material fixed in corrosive-acetic. Creosote clears very slowly but does not produce excess hardening, which is invariably followed by fragmentation of the filamentous forms. An attempt was made to culture *Pseudobilharziella querquedulae* in sterile sheep serum according to the method of Lee and Chu (3) but twelve hours was the maximum time that they could be maintained alive.

DESCRIPTIONS

Genus *Pseudobilharziella* Ejsmont, 1929

Generic diagnosis—Bilharziellinae: Males with elongated filamentous bodies having the anterior portion broader than that in the testicular region; hinder region of the body bearing the testes relatively long and with sides almost parallel; oral sucker and acetabulum well developed and close together; epidermis spined; genital pore lies to the side considerably separated from acetabulum; gynaecophoric canal begins in broader anterior region and extends to beginning of testicular region; testes numerous and fairly regularly arranged; intestine very similar to that of *Bilharziella* and *Gigantobilharzia;* the cecal branches unite in vicinity of genital pore and common cecum follows a zigzag course to almost hinder end of body.

Type species.—Pseudobilharziella kowalewski Ejsmont, 1929.

Pseudobilharziella querquedulae sp. nov. (Plate I)

Specific diagnosis.—Pseudobilharziella.

Male.—Filamentous worms of almost pure white color, measuring 3.7 mm. in maximum length by 150 μ in maximum width. The body is usually depressed dorso-ventrally and the sides are almost parallel throughout most of the length. The cuticle is thin and bears numerous small blunt spines. The oral sucker is terminal and cone shaped with the mouth aperture ventral in position. The anterior body region is long and cylindrical in younger specimens but flattened in older ones. The acetabulum is moderately large, circular in outline with a heavy rim. In younger specimens it is stalked and projects some distance but in older ones it is withdrawn almost to the level of the body surface. In both it bears numerous blunt spines. At about the junction of the first and second fourths of the body there is a lateral expansion of the wall with edges infolded to form a short shallow gynaecophoric canal. Posterior to this the body is almost uniform in width, being squarish in younger specimens and flattened in mature ones. The body termination is flattened, expanded and blunt. The esophagus is a narrow

tube and forms an expanded bulb just cephalad of the acetabulum from which the cecal branches arise. These pass backward to unite in the region of the gynaecophoric canal just caudad of the genital pore. The common cecum follows a zigzag course among the testes almost to the posterior end of the body. In young specimens these curves are somewhat rounded while in older ones they are sharp and set about at right angles or less. Testes vary from about 210 to 240 in number; are somewhat rounded in immature specimens and quite distinct from one another, but in mature specimens they are closely packed. The vas deferens can be traced as a fine tube from the region of the anterior testes to the anterior end of the seminal vesicle just caudad of the acetabulum. The seminal vesicle is a stout convoluted tube of uniform diameter passing backward from near the acetabulum to near the beginning of the gynaecophoric canal. At its hinder end it is continuous with an elongated prostate gland. From this a short ejaculatory duct leads to the genital pore on the ventral surface to the left side of the midline near the beginning of the gynaecophoric canal. A cirrus sac is present. Spermatozoa may be seen in large numbers in the testes, vas deferens, and seminal vesicle especially in living worms where they are quite active.

Body measurements for both mature and immature specimens are as follows: based in each case on the average of eight typical specimens fixed in corrosive-acetic and mounted in Canada balsam:

Immature specimens:—Body 3.6 mm. long by 112 μ in maximum width. Oral sucker 64 μ long by 56 μ in greatest diameter. The acetabulum is situated 375 μ from the anterior end, is 73 μ in diameter and projects 56 μ beyond the body surface. The loosely coiled seminal vesicle begins 37 μ from the base of the acetabulum and extends caudad a distance of 412 μ. The gynaecophoric canal begins 880 μ from the anterior end and is about 375 μ in length.

Mature specimens:—Body about the same average length as in immature specimens but averages about 140 μ in greatest width. The oral sucker is similar to that of the immature worm but the acetabulum is situated 274 μ from the anterior end and projects only slightly beyond the body surface. The seminal vesicle is tightly coiled and begins close to the acetabulum. The gynaecophoric canal begins 678 μ from the anterior end and the seminal vesicle is thus decreased considerably in total length. The portion of the body posterior to the gynaecophoric canal is strap-like and the testes are transversely elongated and more closely packed together than in immature specimens. The common cecum is much convoluted and bends at acute angles.

Female.—Morphological details not known.

Egg.—Not known.

Cercaria.—Unknown or unrecognized.

Type host.—Primary, birds (*Querquedula discors*).
Secondary, unknown.

Type locality.—Long Lake, Marquette, Manitoba, Canada.

Location.—Portal and intestinal veins.

The above description is based on the examination of approximately fifty specimens, both fixed and living, collected from *Querquedula discors*. A single fragmented female was obtained and in general external features resembles the male but is somewhat shorter. More favorable material must be obtained before a detailed description can be given. Repeated attempts to recover eggs from infested hosts have been futile.

During the spring and late fall months only mature specimens are obtained but in summer and early fall a mixture of immature and mature specimens is obtained from adult birds. During the same period only immature worms are found in fledglings. In view of the great difference

in general appearance and relative body measurements between the immature and mature forms it has been considered advisable to describe the minor details of each separately.

The characters of the above described species correspond very closely to those of the genus *Pseudobilharziella* Ejsmont, 1929, in that it possesses an elongated strap-like body, the anterior portion of which is somewhat broader, poorly developed gynaecophoric canal, extending from the genital pore to the anterior testis, well developed oral sucker and acetabulum, long tubular seminal vesicle, and numerous testes in the male.

It also has the following characters in common with the genus *Bilharziella* (Kowalewski, 1896) Looss, 1899 (4).—Elongated tubular seminal vesicle with a well developed cirrus pouch and prostate gland, and also, the cecal branches united at the level of or just posterior to the genital pore. It differs from *Bilharziella polonica* Kowalewski, 1896 (2), in the relative lengths and breadths of the two body regions, in the form of the gynaecophoric canal, also in having a longer coiled seminal vesicle, a large number of testes and spined epidermis.

This species resembles *Pseudobilharziella kowalewski* Ejsmont, 1929 (1), in body shape, length and position of the gynaecophoric canal and the position of the genital pore. Again it differs from the species described by Ejsmont in minor metromorphic characters, in the form of the cirrus sac and prostate gland and cecal branches, which do not unite anterior to the genital pore. It differs from *Bilharziella yokogawai* Oiso, 1927 (6), a closely related form, in that the gynaecophoric canal arises some distance caudad of the acetabulum, the seminal vesicle is longer and more cylindrical, and a cirrus pouch and prostate gland are present. In view of these consistent differences the writer is of the opinion that the above is a previously undescribed species and the name *Pseudobilharziella querquedulae* is suggested.

The assigning of this species to its proper genus is a matter open to considerable question, depending mainly on the validity of the genus *Pseudobilharziella.* In his generic diagnosis Ejsmont sets out the following characters for his genus *Pseudobilharziella.*—Male; the anterior body portion up to the region of the testes is somewhat broader. Suckers present. Epidermis spined? The gynaecophoric canal begins in the broader anterior body region and extends from the pore region to the anterior limit of the testicular region. The intestine as in *Bilharziella* and *Gigantobilharzia* but the union of the intestinal branches lies in front of the genital pore. Testes and end apparatus as in *Gigantobilharzia.* The genital pore lies to the side in considerable separation from the ventral sucker.

Considering the general characters of the digestive and reproductive

systems in the four species mentioned above it would appear that they were cogeneric. The suckers and the testes are similarly located in all, and a well developed seminal vesicle and genital pore opening lateral to the mid-ventral line is also present in each. The union of the intestinal ceca varies slightly in position in different species but this appears to the writer to be a somewhat unreliable character. However, considering the general body form and details of organization the four species fall into two distinct groups, the first of which contains *Bilharziella polonica* and the second group the remaining three. In the genus *Bilharziella* the body is composed of two regions, a narrow anterior one and a broader lanceolate posterior one, the point of union being close to the junction of the second and third fifths of the body. In the genus *Pseudobilharziella* the body is also composed of two fairly distinct regions, the anterior one is broader and relatively short while the posterior region is long, narrow and with almost parallel sides. The union of the two regions in this genus is at about the junction of the first and second fifths of the body.

On this basis the genus *Pseudobilharziella* Ejsmont, 1929, appears to be a valid one, and in the writer's opinion should be retained, at least tentatively, as such. If this is done it undoubtedly should contain the species *Pseudobilharziella querquadulae* sp. nov. and *Bilharziella yokogawai* Oiso, 1927, as well as the type species, because of the striking similarity between all three.

Genus *Ornithobilharzia* Odhner, 1912

ORNITHOBILHARZIA LARI sp. nov. Plate 2

Specific diagnosis.—Ornithobilharzia.

Male.—Stout sickle-shaped worms with the posterior portion of the body curved ventrad, and of a creamy white color. The lateral edges of the body from just caudad of the oral sucker to almost the posterior end are infolded to form a deep gynaecophoric canal which is almost closed ventrally in most specimens, and in which the female is securely held. The average body measurements of ten specimens fixed in formalin and mounted in Canada balsam are as follows: Length 3.72 mm. with a maximum thickness of 490 μ just caudad of the mid-region. Both ends of the body taper somewhat and end bluntly. The cuticle is thick and bears numerous fine blunt spines. The oral sucker is terminal, pointed anteriorly and the mouth aperture is decidedly ventral. It is set off from the body by a slight constriction and the walls are thick. The average length of the oral sucker is 180 μ and width 135 μ. The anterior body region is subcylindrical, short and about 157 μ in thickness. The acetabulum is situated 440 μ from the anterior end and is usually seen as a fungiform structure protruded above the body surface. It measures 247 μ in diameter and its muscle fibres can be traced deep into the body tissue. It is armed with stout blunt spines and the rim is heavy. The body, disregarding the infolded lateral edges, is considerably thickened directly behind the acetabulum but immediately becomes thinner until a uniform thickness of 184 μ is reached and maintained almost to the caudal end. The gynaecophoric canal originates a short distance caudad of the oral sucker and gradually becomes deeper as it passes backward. The edges pass around the stalk of the acetabulum and then curve sharply mesad to almost meet in the

median line. It reaches almost to the posterior tip of the body. The mouth cavity is funnel-like and connects with a narrow esophagus. This in turn connects with a large expanded muscular bulb just cephalad of the acetabulum. From this pharynx-like structure the two cecal branches arise and pass one on each side of the acetabulum stalk. They measure 34 μ in diameter and pass backward following a zigzag course with sharp angles. Their walls bear numerous minute evaginations but there are no anastomoses before the final junction. The common cecum is only slightly convoluted and measures about 262 μ in length and the union takes place 390 μ from the posterior end of the body. Testes are 22–26 in number and are arranged in two irregular rows in the anterior half of the body. Each testis is oval in lateral view and almond shaped in dorsal view with the pointed end directed mesad. The seminal vesicle is free in the parenchyma and is just cephalad of the anterior testis. It is about one-half the size of a testis and is elongated in most specimens. From it a short ejaculatory duct leads to the genital pore on the mid-ventral surface just posterior to the base of the acetabulum.

Female.—Filamentous flattened worms averaging about 2.76 mm. in length by 180 μ in greatest width. The body is widest just caudad of the equatorial region and the hinder portion is bluntly tapered while the anterior portion is more attenuated. Both oral sucker and acetabulum are present, and the ovary is a coiled tube in the anterior half of the body just cephalad of the midbody region. The oral sucker is very similar in shape and position to that of the male and measures 64 μ in length by 52 μ in greatest diameter at the base. The acetabulum appears to be sessile and is situated 247 μ from the anterior end and has an average diameter of 56 μ. The digestive system has a bulbous pharynx-like swelling just cephalad of the acetabulum and from which the cecal branches arise. These are narrow and pass backward to unite a short distance caudad of the ovary. The common cecum follows a zigzag course for a short distance, then increases in diameter and becomes straighter as it passes caudad to end near the posterior body termination. The ovary is a cylindrical tightly coiled body beginning about 468 μ caudad of the acetabulum and, disregarding the total length of the coils, measures 326 μ in length. The receptaculum seminis is round to oval in shape and lies just posterior to the ovary to one side of the median line. The oviduct passes laterad then cephalad past the ovary and connects with the Mehlis' gland a short distance caudad of the acetabulum. The uterus is short and contains a single egg. The genital pore is small and is located about 22 μ behind the acetabulum and is midventral in position. The vitellaria are composed of numerous indistinct transverse follicles which begin directly behind the receptaculum seminis and occupy most of the posterior half of the body.

Egg.—Broadly oval to spherical in shape and measuring, on the average, 67 μ by 60 μ. It has a smooth shell and although a spine may be present it could not be demonstrated.

Cercaria.—Unknown or unrecognized.

Type host.—Primary, birds (*Larus argentatus*), secondary, unknown.

Type locality.—Yarmouth, Nova Scotia, Canada.

Location.—Portal and intestinal veins.

The above description is based on the examination of twenty-two pairs of males and females from the portal and intestinal veins of three specimens of *Larus argentatus* collected in the vicinity of Yarmouth, N. S. The viscerae only of these birds were received and these had been preserved for some time in weak formalin solution. The females were securely held in the gynaecophoric canals of the males and in some cases were recovered only by dissecting away the male. In general the females are considerably shorter than the males but considerable shrinkage due

to fixation was apparent. For this reason stress can not be placed on the absolute measurements of the females and only in a relative way are they of any great value. A number of eggs were observed but internal detail had been destroyed by the preservative.

The general characters of the male bear close resemblance to the generic characters of the genus *Microbilharzia* Price, 1929 (7), in that the gynaecophoric canal arises cephalad of the acetabulum and also in that the testes are relatively few in number. The general body shape and the characters of the male digestive system agree with those of the genus *Ornithobilharzia* Odhner (5). The reproductive organs, the oral sucker and acetabulum of the female are also distinctly those of *Ornithobilharzia.*

The above described species differs widely in body measurements and number of testes from all recorded species known to the writer except *Ornithobilharzia pricei* Wetzel, 1930 (8). It does differ from this species, however, in the shape of the oral sucker, length of the female relative to that of the male, the point of origin of the gynaecophoric canal in the male and the length and diameter of the ovary in the female as well as many other morphological differences. In view of the fact that *Ornithobilharzia pricei* with less than 60 testes is a legitimate species the writer feels that the above species is rightly a member of the Genus *Ornithobilharzia.* As it does not correspond to any species known to the writer it is submitted as being new to science and the name *Ornithobilharzia lari* is suggested.

DISCUSSION

Bilharziella querquedulae was obtained from approximately 60 per cent of specimens of *Querquedula discors* collected in a number of localities in Manitoba, Canada. This bird is a common summer resident in the southern half of the Provence and an uninfested locality was not encountered. The localities studied to the greatest extent were Long Lake, Marquette, and a lagoon off Pelican Lake, Ninette, Manitoba. In both places dermatitis-producing non-human schistosome cercariae are abundant from both *Lymnaea stagnalis* var. and *Stagnicolis palustris.* The writer has collected infested fledgling birds of about six weeks of age from both localities and also recovered immature as well as mature worms from adult specimens of *Querquedula discors.* In view of this it is reasonable to suppose that *Pseudobilharziella querquedulae* is the adult stage of one of the local schistosome cercariae.

Specimens of *Larus argentatus* are common about the larger Manitoba lakes and rivers but up to the present it has been impossible to obtain local material for examination and it is not known if local birds are infested with *Ornithobilharzia lari.*

ACKNOWLEDGMENTS

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Legend

ac.	Acetabulum.
cb.	Cecal branch.
cc.	Common cecum.
e.	Egg.
gc.	Gynaecophoric canal.
gp.	Genital pore.
o.	Ovary.
od.	Oviduct.
os.	Oral sucker.
p.	Prostate.
rs.	Receptaculum seminis.
s.	Spermatozoa.
sv.	Seminal vesicle.
t.	Testes.
v.	Vitellaria.
vd.	Vas deferens.

PLATE I

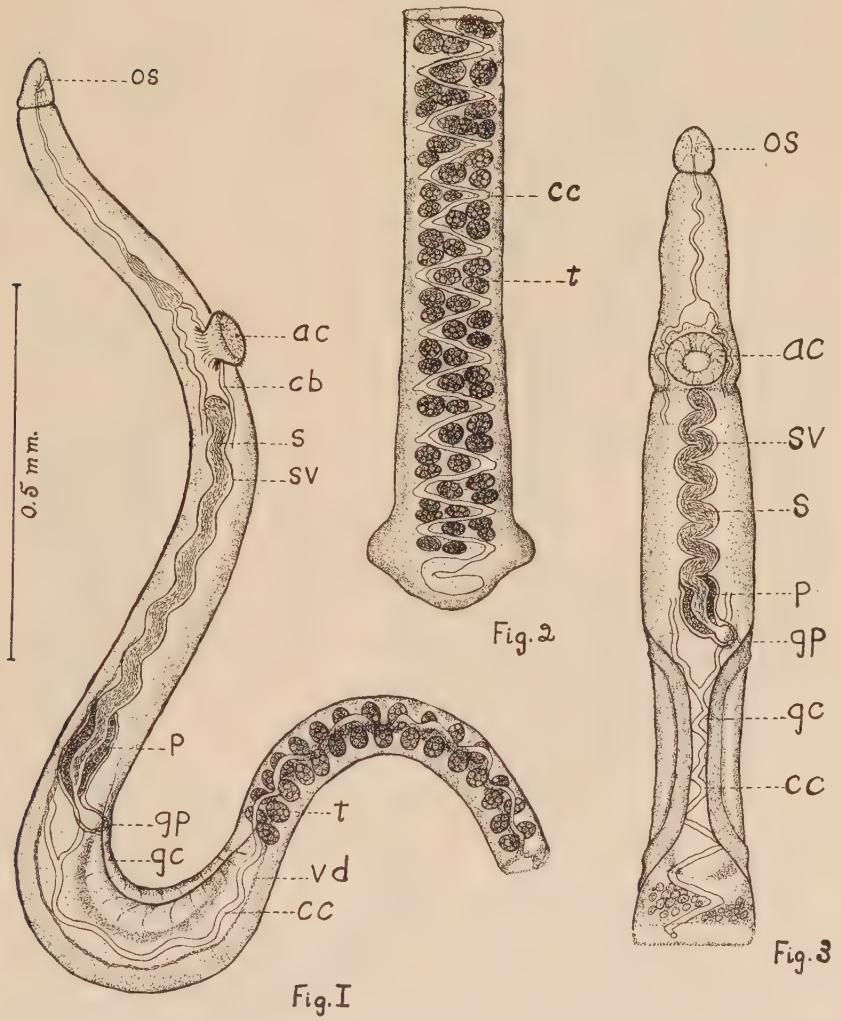


Fig. 1. *Pseudobilharziella querquedulae.* Anterior portion of immature male.
Fig. 2. *Pseudobilharziella querquedulae.* Male, posterior portion of body.
Fig. 3. *Pseudobilharziella querquedulae.* Anterior portion of mature male.

PLATE II

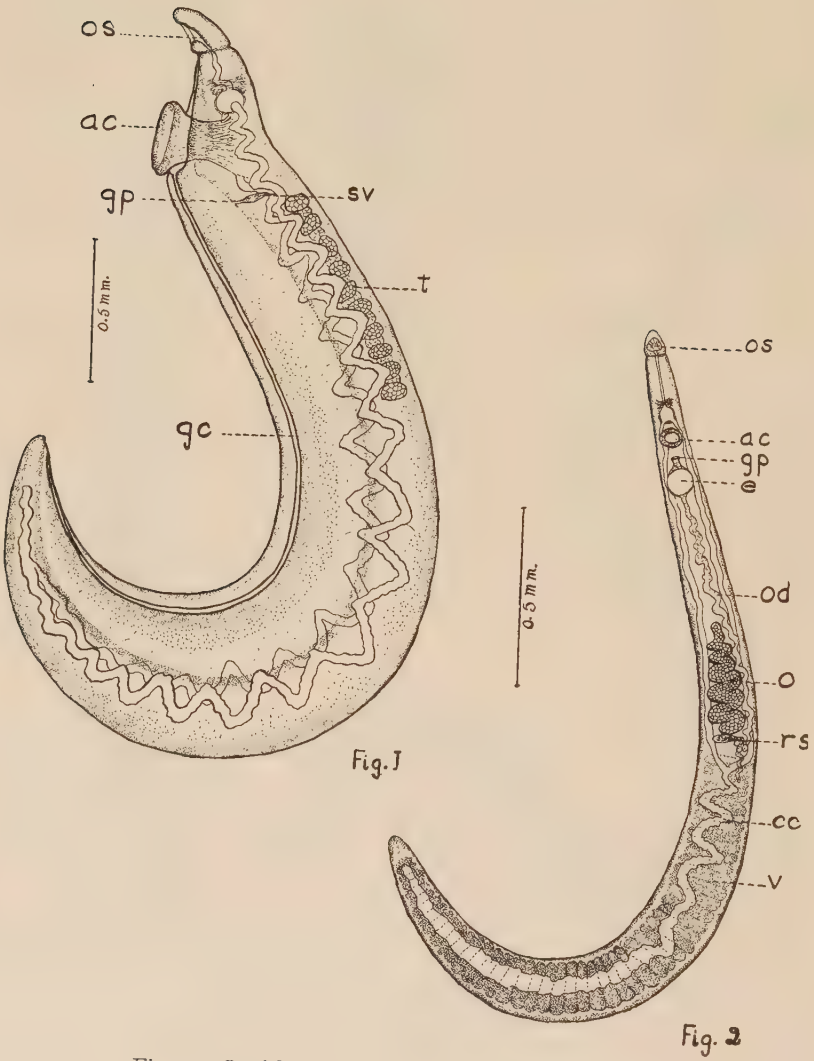


Fig. 1. *Ornithobilharzia lari.* Male, lateral view.
Fig. 2. *Ornithobilharzia lari.* Female, ventral view.

ON SOME NEW AND KNOWN MYXOSPORIDIA OF THE FISHES OF ILLINOIS*

PAUL A. MEGLITSCH

The present paper is based upon the microscopical examination of two hundred fishes of Illinois for Myxosporidia. These fishes were collected between February and August, 1935, from southern and east-central parts of the state. The species of fish, Myxosporidia and the organs in which the protozoans were found are summarized in the following table.

Fishes examined	Number examined	Number infected	Organ infected	Myxosporidia found
Ameiurus melas	5	..	.....	.....
Ameiurus nebulosus	3	..	.....	.....
Anguilla bostoniensis	2	1	kidney	*Myxidium illinoisense* n. sp.
Aplodinotus grunniens	27	2	gall bladder	*Myxidium aplodinoti*
		2	gall bladder	*Chloromyxum* sp.
		1	gall bladder	*Myxidium aplodinoti* and *Chloromyxum* sp.
Apomotis cyanellus	3	..	.....	.....
Campostoma anomalum	4	1	gills	*Henneguya crassicauda*
Carpiodes cyprinus	1	1	gills	*Myxosoma rotundum* n. sp.
Chaenobryttus gulosus	2	..	.....	.....
Cynoperca canadensis grisea	1	..	.....	.....
Cyprinius carpio	1	..	.....	.....
Dorosoma cepedianum	1	..	.....	.....
Ericymba buccata	3	..	.....	.....
Erimyzon succetta	3	..	.....	.....
Eupomotis gibbosus	2	..	.....	.....
Helioperca incisor	9	1	stomach wall	*Myxobolus* sp.
		2	gall bladder	*Chloromyxum trijugum*
		1	gall bladder	*Chloromyxum* sp.
Huro salmoides	1	..	.....	.....
Hyborhynchus notatus	3	..	.....	.....
Ictalurus anguillae	4	..	.....	.....
Ictalurus furcatus	34	19	gall bladder	*Myxidium kudoi* n. sp.
		1	gall bladder	*Henneguya limatula* n. sp.
Ictalurus punctatus	24	6	gall bladder	*Myxidium bellum* n. sp.
		1	skin	*Henneguya exilis*
		2	gills	*Henneguya exilis*
		1	gall bladder	*Henneguya limatula* n. sp.
Ictiobus bubalus	3	1	gall bladder	*Chloromyxum* sp.
Lepibema chrysops	3	..	.....	.....
Lepisosteus osseus	1	..	.....	.....
Lepisosteus platostomus	1	..	.....	.....
Micropterus pseudoplites	1	..	.....	.....
Minytrema melanops	1	1	mesentery	*Myxobolus* sp.
Notemigonus crysoleucas	1	..	.....	.....
Opladelus olivaris	18	7	gall bladder	*Myxidium* sp.
		1	gall bladder	*Myxidium* sp. and *Chloromyxum* sp.
Perca flavescens	2	..	.....	.....
Pimephales promelas	3	..	.....	.....
Polyodon spathula	13	2	gall bladder	*Myxidium* sp.
Pomolobus chrysochloris	1	..	.....	.....
Pomoxis annularis	4	..	.....	
Pomoxis sparoides	10	2	intestinal wall	*Myxobolus intestinalus*
		4	gall bladder	*Chloromyxum trijugum*
Semotilus atromaculatus	4	..	.....	.....
Xenotis megalotis	1	1	gall bladder	*Chloromyxum trijugum*
Total	200	60		

* Contributions from the Zoological Laboratory of the University of Illinois, No. 508. I wish to express my thanks to Professor R. R. Kudo, at whose suggestion this study was undertaken, for his guidance and assistance in many ways. I am indebted to Dr. D. H. Thompson, of the Illinois State Natural History Survey, who kindly provided me with the fishes collected in the Ohio River.

Myxidium illinoisense n. sp. (Figs. 1–4)

Habitat. This myxosporidian was found in the connective tissue of the kidney of *Anguilla bostoniensis.* One of the two examined contained numerous small cysts which were preserved in four per cent formaldehyde. Ohio River, Shawneetown.

Vegetative form. Examination of the infected kidney in sectioned preparations revealed that the cysts were usually surrounded by a very thin layer of connective tissue (Fig. 1), which thickness, however, varied a little. Although certain cysts were of an elongate, oval, or irregular shape, the majority were flattened spheres. One of the most extremely elongated cysts measured 384 μ in length by 12 μ in width at the narrowest point. In addition, this cyst was quite irregular in shape for at one point a branch 100 μ long and 27 μ wide protruded from it. This may have been due to fusion of two trophozoites. The largest cyst found in sections was 658 μ long by 240 μ wide, while the smallest measured 79 μ by 21 μ.

The more or less vacuolated cytoplasm does not show any differentiation and is filled with numerous nuclei and mature and developing spores. Even the smallest cysts contained some developing sporoblasts (Fig. 4). As is usually the case the mature spores are tightly packed in the central portion of the cyst surrounded by younger spores which are located in the peripheral area. The trophozoite is polysporous, but each sporoblast seems to develop into a single spore, which is an unusual feature of a polysporous Myxidium.

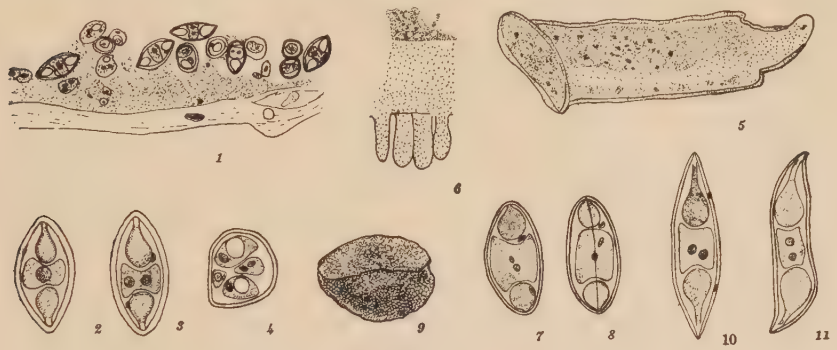
The spore (Figs. 2 and 3) is fusiform in front and side views. It is bluntly pointed at each end, but varies somewhat in size and shape. The shell is rather thick, and the sutural line is distinct, although the ridge is not greatly developed. The valves bear 7 or 8 striations, which are distinctly visible in unstained spores. The majority of the spores are straight, but some may be slightly curved in an S-shape.

The sporoplasm is small and contains a single large nucleus, or two smaller nuclei. The polar capsules are pyriform. The capsulogenous nuclei remain visible for some time after the spore has matured, as has been reported in many species.

The dimensions of the fresh spores are 12.7 to 15.3 μ in length, and 7.6 to 9.3 μ in width. The polar capsules are 3.4 to 6.1 μ in length. The average spore measures 13.9 μ in length, 8.3 μ in breadth, with polar capsules 4.3 μ in length.

Identification. Of the 61 known species of Myxidium (Kudo, 1933, 1934), three species have been reported from eels. One of these, *Myxidium giardi,* was described from the kidney of *Anguilla vulgaris* by Cépède (1906). The spores of *M. giardi* are entirely different from those of the present form in shape, being much wider and with more

rounded extremities. The two other species reported from eels of Japan, *M. uchiyami* Fujita, 1927, and *M. anguillae* Ishii, show differences in either size or shape of the spore. It seems proper, therefore, to consider the species described above as new, and the name *Myxidium illinoisense* n. sp. is proposed for it.



1–4. *Myxidium illinoisense* n. sp. 1. Section of a cyst, × 590.
2, 3. Stained spores, × 1680. 4. A young spore, × 1680.
5–8. *Myxidium kudoi* n. sp. 5. A living trophozoite, × 60. A portion of periphery of trophozoite in section, × 1680. 7, 8. Stained spores, × 1680.
9–11. *Myxidium bellum* n. sp. 9. A stained trophozoite, × 190. 10, 11. Stained spores, × 1680.

Myxidium kudoi n. sp. (Figs. 5–8)

Habitat. This myxosporidian was found in the gall bladder of *Ictalurus furcatus,* the blue or Fulton catfish. The host fishes were taken from the Ohio River at Shawneetown and Rosiclaire, where infected fishes were found between May 1 and August 1, 1935. Nineteen out of 32 fishes examined harbored this organism.

Vegetative form. The trophozoites are quite large, reaching 1 mm. in length. They are ovoid to elliptical, or irregular in outline. In the smaller forms, the length seldom exceeds 1.5 times the breadth. They are extremely flattened; one of the larger sectioned specimens which measured 761 μ in length was about 43 μ in thickness. Because of the thinness of the body, the trophozoites are often seen rolled up at one or both ends (Fig. 5). Living trophozoites are yellowish in color and granulated. In many individuals a distinct hyaline ectoplasmic zone could be recognized. No movement of the trophozoites was noted.

In sectioned preparations, the trophozoites are distinctly divided into ectoplasm and endoplasm. The endoplasm is highly alveolated, and the interalveolar cytoplasm appears granular. Near the ectoplasm the alveoli become smaller in size and give rise to a more compact, coarsely granular zone, containing many nuclei. The spores lie in the inner alveolar part of the endoplasm.

The ectoplasm is differentiated into three zones (Fig. 6). The inner zone is about 7.5 to 8.5 μ in thickness. The middle zone, about 1 to 1.5 μ thick, stains more deeply than the inner zone. The outer zone is made up of a large number of small hair-like projections, 8 to 10 μ long, staining in the same way as does the middle zone. The exact significance of these projections is not known, although similar ectoplasmic structures have been described in *Myxidium lieberkühni* (Cohn, 1896) and *Myxidium phyllium* Davis (1917), etc. In the latter form, they are said not to be permanent structures, being formed only after the parasite has been removed from the host organ and has been on the slide for some time. In the present form the trophozoites have always showed these filaments, even when several parasites were studied at a fish market, where the time between the removal of the gall bladder from the live fish, and the actual observation of the trophozoite on the slide did not exceed five minutes. Polysporous and disporoblastic.

Spore. The spore (Figs. 7 and 8) is bluntly rounded at the extremities with two subspherical polar capsules. The shell is relatively thin, and presents a rather distinct sutural line, but the ridge is not developed. The valves are striated longitudinally, about seven striae occurring on each valve. The sporoplasm usually almost fills the cavity of the spore between the capsules, but may be drawn away from the capsules slightly. It contains one or two nuclei. The capsulogenous nuclei are persistent, and one rarely finds a spore in which one of them is obscured. The shell nuclei are rarely observed in the fully developed spore.

A comparison of the amount of shrinkage in formaldehyde and Carnoy's fluid was made on the spores of this species. Two series of 100 spores each were measured in distilled water after fixation in four per cent formaldehyde, and one such series in distilled water after fixation in Carnoy's and washing in alcohol. Dimensions recorded were: Formaldehyde, length of spore, 10.1 to 11.92 μ and 9.3 to 11.9 μ; breadth of spore, 4.2 to 6 μ for both series; diameter of polar capsules, 2.5 to 3.4 μ for both series. Carnoy, length of spore, 8.5 to 11 μ; breadth of spore, 3.4 to 6 μ; diameter of polar capsules, 2.5 to 3.4 μ. Thus shrinkage was less in the formaldehyde solution than in Carnoy's. Kudo (1921) studied the effect of a number of fixatives on *Leptotheca ohlmacheri.* He found that formaldehyde caused a greater amount of shrinkage than several other fixatives, but Carnoy was not tried.

In order to compare the shrinkage induced by various mounting media in which spores were studied, a series of slides fixed with 4 per cent formaldehyde were stained in Heidenhain's haematoxylin, and 50 spores were measured in distilled water, cedar wood oil, and Canada balsam. For these measurements a mechanical stage was used, and the same spores measured in each medium. The results were:

Mounting Medium	Length (μ)	Spore breadth (μ)	Diameter of polar capsules (μ)
	Ave.	Ave.	Ave.
Distilled water ...	10.2 – 11.9 (10.54)	4.2 – 6.0 (4.75)	2.5 – 3.5 (2.66)
Cedar wood oil ..	9.3 – 11.0 (9.62)	2.3 – 5.0 (3.85)	2.5 – 3.4 (2.51)
Canada balsam ..	9.3 – 10.1 (9.52)	2.3 – 5.0 (3.60)	2.5 – 3.4 (2.35)

The results show that for the present myxosporidian there is as much or greater difference in the amount of shrinkage of the spore induced by the change from distilled water to balsam as that induced by the killing solution used. In order to make exact comparisons of the size of the spores, not only the same fixative, but the same medium should be used for observation. Kudo (1921 and 1934) suggests that a new species should be described after studying the spores in fresh as well as fixed and stained conditions, and, if possible, the fixed vegetative form. He has also suggested that, in descriptions of new species, the actual mention of the killing solution and mounting medium would be conducive to more accurate taxonomic considerations.

Identification. Sixty-one species of Myxidium are known to date (Kudo, 1933 and 1934). A comparison of the shape of the spore of the coelozoic species with respect to curved or pointed ends, straightness of sutural line, and the position of the opening of the polar capsules, with ratio of length to breadth, and ratio of the length of the spore to the diameter of the polar capsules shows that only one, *Myxidium therapon* Johnston and Bancroft (Kudo, 1920) is very similar to the present form. The trophozoites of *M. therapon,* however, are described as being from 2 to 12 mm. in length which is far greater than the form under consideration, and there is in addition a difference in the shape of the spore, the present form having more rounded extremities than the spores of *M. therapon.* It therefore seems proper to consider the form here described as a new species, for which the name *Myxidium kudoi* n. sp. is proposed, in honor of Dr. Kudo, who has contributed a great deal to our knowledge of Myxosporidia.

Myxidium bellum n. sp. (Figs. 9–11)

Habitat. This protozoan was found in the gall bladder of *Ictalurus punctatus,* the channel catfish. The infected host fishes came from the Ohio River at Shawneetown and Rosiclaire and were examined at Urbana. Six of seventeen fishes from Shawneetown were infected, and two of five from Rosiclaire harbored the spores in the gall bladder.

Vegetative form. The trophozoites (Fig. 9) found in the gall bladder of infected catfishes were somewhat like those of *Myxidium kudoi.* They were, however, oval in general outline, greatly flattened, and leaf-like. The larger forms, which are frequently irregular in *M. kudoi,* are

more smoothly outlined in the present form. There is the same tendency toward a curling of the vegetative form in this species that was noted in *M. kudoi.*

The endoplasm is alveolated, and filled with granules. The spores lie in the endoplasm, frequently towards the center. The differentiation of the ectoplasm is similar in this form to that described for *M. kudoi,* manifesting the same fine hair-like filaments. The ectoplasm is distinctly hyaline. The form is polysporous, and each sporoblast develops into two spores.

Spore. In side view, the spore is elongate and cylindrical, ending in sharply pointed extremities which are recurved into an S-shape. The shell valves carry six or seven striae which are recognized with difficulty. The valves are uniformly thin. The sutural line is distinct and somewhat curved. In front view (Fig. 11) the spore is straight and lenticular, with sharply pointed extremities.

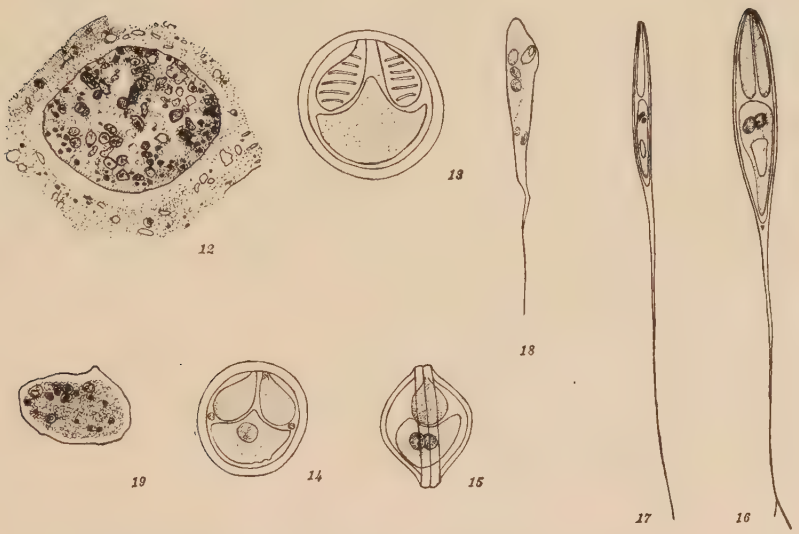
The polar capsules are comparatively large, with a long slender neck, and a widely pyriform posterior part. The capsulogenous nuclei are not as persistent as is the case with the two species described above, although in many cases they were found. Occasionally the shell nuclei may be found adhering to the inner wall of the spore, or lying in the wall itself. The somewhat granular sporoplasm fills the cavity of the spores between the capsules and always contains two small nuclei lying close together. In no case was a uninucleate sporoplasm seen.

The dimensions of the spore, measured in distilled water after killing in four per cent formaldehyde, are 15.5 to 17.5 μ in length, 4 to 5 μ in breadth, and the polar capsules measure 6 to 7 μ in length, and 2.5 to 3 μ in breadth.

Identification. Of the 61 known species of Myxidium (Kudo, 1933 and 1934) only a few have a spore with the characteristic shape seen in this organism. None of these are identical with the form under consideration in all particulars. The two which most closely resemble the present form are *Myxidium lieberkühni* Bütschli and *M. contortum* Fantham. The former is found in the urinary bladder of *Lota lota* and *Esox lucius* and has been reported from the United States and Canada. The spores are somewhat larger and straighter in this species than in the present form. The spores of *M. contortum* are about 50 per cent larger than those of the parasite from the catfish. Therefore, I am led to believe this to be a new species and propose to name it *Myxidium bellum* n. sp.

Myxosoma rotundum n. sp. (Figs. 12–15)

Habitat. This myxosporidian was found in the connective tissue of the gill filaments of *Carpiodes cyprinus.* A single infected fish was obtained from the Embarass River 3 miles north of Villa Grove on May 2,



12–15. *Myxosoma rotundum* n. sp. 12. Section of a cyst, × 190. 13. A spore treated in Lugol's solution, × 1680. 14, 15. Stained spores, × 1680.

16–18. *Henneguya limatula* n. sp. 16, 17. Stained spores, × 1680. 18. A developing spore, × 1210.

19. A living *Chloromyxum trijugum*, × 190.

1928, and preserved in four per cent formaldehyde. There were numerous small, round, whitish cysts in the gill filaments.

Vegetative form. The almost spherical cysts were small, and measured about 0.5 mm. in diameter. Each cyst was distinctly contoured, and protruded from the surface of the filament. The cysts numbered 58 in all, 23 and 25 being on the left and right gills respectively. These were distributed on all the gills, and on both anterior and posterior surfaces. As seen in the preserved condition, they were white to cream-colored. In sections (Fig. 12), no flattening of the cysts could be seen. A broad zone of compact and granular ectoplasm surrounds the highly reticulated endoplasm, in which numerous nuclei, developing pansporoblasts and mature spores were located.

Spore. The spore is circular or subcircular in front view (Figs. 13 and 14), and lenticular to oval in side view (Fig. 15). The shell valves are rather thick and smooth. The sutural line is distinct, and the ridge is well developed, showing two distinct lips with a groove between them. The polar capsules are broadly pyriform, with a short neck. The capsulogenous nuclei are frequently seen. The sporoplasm occupies the posterior part of the spore. It contains a single large nucleus, or more rarely, two large nuclei. The sporoplasm is compact and granular. It contains no iodinophile vacuole. The dimensions of the spore were: 12 to 14 μ in length, 11 to 13 μ in breadth, 7 to 8.5 μ in thickness. The

polar capsules were 6 to 7 μ in length and 3 to 4 μ in breadth. The measurements were made from the spores preserved in four per cent formaldehyde and suspended in distilled water.

Identification. Compared with the known species of Myxosoma, numbering 27 (Kudo, 1933, 1934), the present form appears to be different from any one of them in one or more distinctive characters. Only one of them, *Myxosoma ovalis* Davis (Davis, 1923), found in the gills of *Ictiobus bubalus* and *Megastomatobus cyprinella* (=*Ictiobus cyprinella*) is very similar to the present form. The spores of this species measure 15 to 17 μ in length, 15 μ in breadth, and 11 μ in thickness. The capsules are 8 to 9 μ in length and 6 μ in breadth. The polar capsules are relatively much larger, and the spore is larger in all dimensions than the present form. There is a further difference in host species. For these reasons the organism here described is considered as a new species and the name *Myxosoma rotundum* n. sp. is proposed.

Henneguya limatula n. sp.

Habitat. This protozoan was found in the gall bladder of *Ictalurus punctatus* and *Ictalurus furcatus,* collected in the Ohio River at Shawneetown and Rosiclaire and brought to Urbana on ice. One *I. furcatus* and one *I. punctatus* were infected; the hosts were from Shawneetown and Rosiclaire respectively.

Vegetative form. The vegetative form of this parasite has not been observed.

Spore. The spore (Figs. 16 and 17) is elongate, with slightly attenuated anterior end tapering gradually from the broadest part, which is about one third of the distance from the anterior end. There is a very long slender posterior process which shows a bifurcation in a few cases. This process gradually tapers to a sharp point. The shell valves are quite thin and smooth. The sutural line and sutural ridge are indistinctly visible. The sporoplasm is compact and granular and fills the posterior part of the spore. Besides two small nuclei, it contains a large iodinophile vacuole staining cherry red in Lugol's solution and remaining unstained in haematoxylin. The vacuole is sometimes divided into two parts. In every case, the two nuclei lie anterior to the iodinophilous vacuole. They may vary somewhat in size. The long and slender polar capsules taper slightly at the anterior end of the spore. They do not completely fill the anterior part of the spore cavity. The capsulogenous nuclei are visible in very few mature spores.

The dimensions of the spores were: 13 to 17 μ in length, 5 to 6 μ in breadth; posterior process 27 to 37 μ in length; the polar capsules 6.5 to 8 μ by 1.5 to 2 μ. These dimensions were taken from spores suspended in distilled water after fixation in four per cent formaldehyde.

Identification. A study of the previously described species of Henneguya shows that coelozoic forms are few in number. Of 54 reported species (Kudo, 1933 and 1934) only 5 are coelozoic. Most of these are very broad, with the spore almost circular, and with the posterior process broad and blunt. *H. mictospora* Kudo (Kudo, 1920) is more like the present species in shape, but there are distinct striations on the valves of that species, as well as a broader form, and shorter polar capsules. For these reasons, the form under consideration is believed to be a new species, and the name *Henneguya limatula* n. sp. is proposed for it.

Chloromyxum trijugum Kudo. (Fig. 19)

Habitat. This myxosporidian was originally reported by Kudo (1920) as an inhabitant of the gall bladder of *Lepomis megalotis.* Jameson (1931) found it in the same organ of *Pomoxis sparoides* in California. During the course of this study, it was found in the gall bladder in the only *Xenotis megalotis* (= *Lepomis megalotis*) studied, and in addition, four of ten *Pomoxis sparoides* and two of nine *Helioperca incisor* studied harbored the parasite.

Vegetative form. Both Kudo (1920) and Jameson (1931) describe the trophozoite of *Chloromyxum trijugum.* Kudo found monosporous, disporous and polysporous forms, the cytoplasm of which was highly transparent and was divided into a hyaline ectoplasm and alveolated endoplasm. They were very polymorphic, and actively produced pseudopodia. Fixed preparations suggested the occurrence of plasmotomy. There was a great size variation in his material, the extremes being 10 by 14 μ and 300 by 50 μ. Jameson found only polysporous forms, but these, too, presented a considerable size variation.

In *Helioperca incisor* polysporous trophozoites were found only. These (Fig. 19) varied considerably in size, as reported by Kudo and Jameson. The largest form observed was 280 μ by 250 μ, and the smallest 120 μ by 60 μ. In all respects except the active movement of the organism, they agreed with the description by Kudo. These forms were very sluggish, and their movement was barely perceptible, but this was probably because the host fish had died some time before the gall bladder was opened.

Spore. Kudo (1920) described the spores as generally circular in front view and oval in side view. The shell is thick and the sutural ridge is straight and distinct. Each valve has a thick straight or zig-zag ridge which runs parallel to the sutural ridge, producing a group of three separate ridges running around the spore, which characteristic suggested the name. Short ridges run toward the center of the valves from the outer ridges. Four pyriform polar capsules are of slightly different sizes. There are two nuclei in the sporoplasm. Jameson (1931) found that the spores from *Pomoxis sparoides* agreed with Kudo's description.

A comparison of the spores from the three species of host fishes show that they are all of approximately the same size and structure. The dimensions of the living spores of *Chloromyxum trijugum* given by Kudo were: length, 8 to 10 μ; breadth, 8 to 10 μ; thickness, 5 to 7 μ; polar capsules, 3 to 5 μ by 2 to 3 μ. The average dimensions of 50 spores from the gall bladder of *Helioperca incisor* were: length, 9.4 μ; breadth, 8.5 μ; thickness, 6.8 μ; polar capsules, 3.8 by 2 μ.

Henneguya crassicauda Kudo

Habitat. This protozoan was found in the gills of *Campostomum anomalum.* It was previously described from cysts in the fins and integument of the same host fish by Kudo (1934). One of four fishes examined had a single cyst of *Henneguya crassicauda* in the gills, attached to a gill filament. No other cysts were found. Camp Creek.

Vegetative form. According to Kudo (1934) the cysts found in the epidermis or connective tissue in the fins or in the subepidermal connective tissue of the integument of the body. The cysts were 200 to 400 μ by 100 to 250 μ. The peripheral cytoplasmic zone appeared to be radially striated. It was disporoblastic. The cyst found in the gills was somewhat larger than those described by Kudo, measuring a little over 500 μ in diameter.

Spore. As described by Kudo, the spore is oval, oblong, or subcircular in front view and broadly fusiform in side view. The shell is moderately thick and smooth, with a prominent sutural ridge and fairly distinct sutural line. The posterior appendage is independent of the shell, expanding into a cup-like anterior part, in which the posterior part of the spore fits. The appendage tapers gradually to a blunt posterior end. The dimensions of the spore reported were: length, 12 to 14.5 μ; breadth, 8.5 to 10.5 μ; thickness, 6 to 7 μ; appendage, 40 to 55 μ by 3.5 to 4.5 μ; polar capsules, 5 to 6 μ by 3 to 3.5 μ.

The spores taken from the cyst on the gill measured: length, 10.9 to 13.5 μ; breadth, 9 to 9.6 μ; thickness, 7.3 μ; appendage, 20 to 40 μ long; capsules, 5.1 to 6.8 μ by 2.6 to 3.9 μ. Thus the appendage was much shorter than that described by Kudo. The capsulogenous nuclei appear to persist long after the spore has been fully formed. The shell nuclei were rarely observed, but when found were pressed closely to the inner surface of the shell.

SUMMARY

1. A report of the results of the examination of 200 fishes of Illinois, belonging to 37 different species, is given.

2. Five new species of Myxosporidia are described.

3. Observations on two known species are given.

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TWO NEW SPECIES OF TREMATODES, A RENIFER *LECHRIORCHIS SECUNDUS* N. SP. FROM *NATRIX S. SIPEDON* AND A DICROCOELID *DICROCOELIUM PROXILLICENS* N. SP. FROM *KAKATOE SULPHUREA*

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Lechriorchis secundus n. sp. (Fig. 1.)

Body elliptical, dorsally oval, ventrally flattened, slightly attenuated anteriorly, narrower behind, 6.09 ± 0.182 mm. in length by 1.981 ± 0.2 mm. in width. Type specimen 6.053 mm. by 1.781 mm. Cuticula covered with spines. Oral sucker subterminal, measuring 0.536 ± 0.027 mm. in diameter. Acetabulum anterior to middle of body 1.636 mm. from anterior end and 1.037 mm. from oral sucker. Acetabulum is 0.518 ± 0.036 mm. by 0.545 ± 0.036 mm. Prepharynx short. Type specimen pharynx 0.29 mm. in length. Esophagus 0.363 mm. in length. Intestinal ceca extend posteriad to region of testes. Right intestinal cecum extends 2.781 mm. in length and reaches middle of right testis. Tip of left intestinal cecum extends posteriad beyond left testis. Lobed testes, obliquely situated to long axis of body, a line passing diagonally through them crosses median line one-third from posterior end. Genital pore median just posterior to bifurcation of intestinal ceca. Cirrus pouch as in the genus, large, narrow before, broad behind, 0.572 ± 0.027 mm. in length, extending from genital pore to right anterior quadrant of acetabulum which it overlaps.

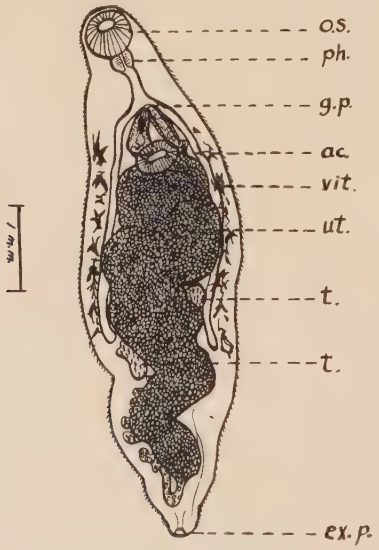

Fig. 1. Ventral view of *Lechriorchis secundus* n. sp.

Abbreviations

o. s., oral sucker; ph., pharynx; g. p., genital pore; ac., acetabulum; t., testis; ov., ovary; ut., uterus; vit., vitellaria; ex. p., excretory pore.

Excretory pore, distinct, at caudal end; excretory tubes, cephalad of ceca on either side, clearly defined.

Spherical ovary near border of right posterior quadrant of acetabulum and difficult to make out because of numerous eggs in expanded uterus. Vitellarian follicles, small and branched, lateral, in middle third of body extending from level of genital pore or region of the acetabulum to near end of ceca. Uterine tube coils posteriad between testes nearly to caudal end then turns and ascends fairly straight. Ascending limb four to five times as broad as descending limb, anterior of the testes becoming enlarged until completely fills inter-cecal space. Metraterm, glandular, muscular, distinctly separated from cirrus sac. Eggs numerous, light brown, measuring 36 μ–44 μ × 20 μ–22.5 μ.

Host.—*Natrix s. sipedon* (L.). Common water snake.

Habitat.—Oviduct.

Locality.—Philadelphia (Penn., U. S. A.) Zoological Garden.

Type specimen.—U. S. N. M. Helm. Coll. No. 9017.

Paratypes.—U. S. N. M. Helm. Coll. No. 9018.

Distribution of Host.—United States: Northeast from the Atlantic to Nebraska and Texas.

P. Z. G. No. 10571.

In general morphological features this new species is closely related to *Lechriorchis primus* Stafford, 1905, type of the genus, from *Thamnophis sirtalis,* hence the name for it. However, in the type species the acetabulum is larger than the oral sucker. In this new species the acetabulum is more nearly equal to the oral sucker in size yet still within the limits of the generic diagnosis. In the type the ceca stop short of the oval testes. In this new species they extend to the middle of or beyond the diagonally placed lobed testes. The latter are strongly oblique in this one. In addition the ova of the type are 52 μ–55 μ × 26 μ–29 μ; in this new species the ova are 36 μ–44 μ × 20 μ–22.5 μ in size. This also differs in length, habitat and host.

Redescriptions and figures by Price, 1936, of Stafford's original types *L. primus* Stafford, 1905, and *Zeugorchis aequatus* Stafford, 1905, will help students of the Reniferinae.

Dicrocoelium proxillicens n. sp. (Fig. 2.)

Body leaf-like, dorso-ventrally flattened, as in the majority of dicrocoelids broadens into a shoulder anteriorly, tapers posteriorly. Length 4.272 ± 0.6 mm., width 2.208 ± 0.2 mm. at the widest part. Type specimen is 4.363 mm. × 2.254 mm. Oral sucker, subterminal, width 0.545 ± 0.036 mm., length 0.481 ± 0.027 mm. Acetabulum, pre-equatorial, 0.945 mm. from anterior end; anterior-posterior diameter 0.563 ± 0.018 mm., dextro-sinister diameter 0.663 ± 0.027 mm. Pharynx of the type specimen 0.143 mm. in diameter. Esophagus, short, length 0.181 mm. not clearly evident. Intestinal ceca ramify right and left around outside of testes from bifurcation immediately behind pharynx. Their termination not evident. Testes, pre-equatorial, on either side of and in zone of posterior half of acetabulum. Anterior-posterior diameter 0.426 ± 0.045 mm., dextro-sinister diameter 0.554 ± 0.1 mm. Genital pore midway between oral sucker and acetabulum just posterior of intestinal bifurcation in some, anterior of intestinal bifurcation and ventral of pharynx in others. In the latter approximately 0.272 mm. from oral sucker. Cirrus-sac extending from genital pore to right anterior quadrant of acetabulum which it overlaps. Length 0.572 ± 0.063 mm., width 0.272 ± 0.054 mm. Cirrus within coiled, muscular.

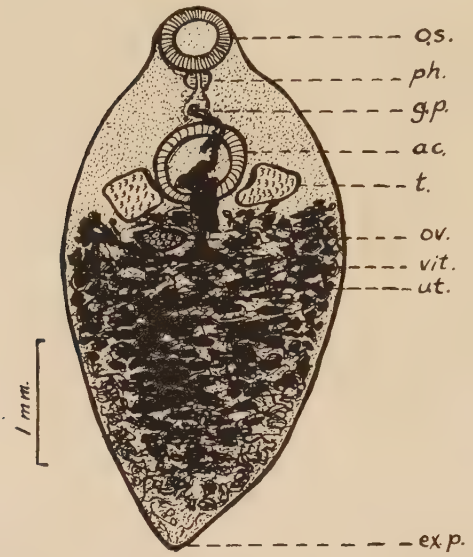

FIG. 2. Ventral view of *Dicrocoelium proxillicens* n. sp.

Ovary, pre-equatorial, post-testicular, lens-shaped, long axis lying lateral. Slightly mediad of right testis. Long axis 0.463 ± 0.064 mm. Short axis 0.17 mm. ± 0.063 mm. Vitellaria, lateral, few in number, in broadest part, i.e., in middle third, of body. Field extends from posterior border of testes 1.09 mm. posteriad and is 0.363 mm. broad. Shell-gland not clearly evident. In paratype seen to be just mediad and slightly posteriad of ovary. Lateral vitelline ducts clearly defined making a line across the middle of the field. Uterus, post-acetabular, its ramifying multiple folds fill the entire posterior half of the body and extend to the margins. Metraterm, thin-walled, dorsad and to left of cirrus sac but parallel with it. Eggs, in metraterm of uterus opaque, others tan, measure 48 μ–56 μ × 24 μ–30 μ. Commonly 52 μ × 30 μ.

Host.—*Kakatoë sulphurea.* Lesser Sulphur Crested Cockatoo.
Habitat.—Bile duct.
Locality.—Philadelphia (Penn., U. S. A.) Zoological Garden.
Type specimen.—U. S. N. M. Helm. Coll. No. 9019.
Paratypes.—U. S. N. M. Helm. Coll. No. 9020.
Distribution of Host.—Moluccas.
P. Z. G. No. 10,458.

This species is closely related to *Dicrocoelium illicens* Brn. 1901 from *Rhamphastus* sp. But *D. illicens* averages 6 mm. in length and is slightly different in shape. It is in the size, shape and position of the testes that they differ most however. *D. illicens* has larger, differently arranged testes that extend into the zone of the anterior half of the acetabulum while this new species has smaller testes that lie within the zone of the posterior half of the acetabulum. Other than slight variations in the distribution of the vitellaria the uteri differ most in the feminine system. In *D. illicens* there is a definite clear zone surrounding the

uterus. In this new species the ramifications of the uterus extend to the margins of the body. And, each is parasitic in a different host.

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ON A COENURUS FROM THE BRAIN OF A MONKEY*

J. H. Sandground

The purpose of this paper is to record the occurrence of a coenurus (a polycephalous larva of a member of the taeniod genus *Multiceps* Goeze, 1782) on the brain of a spotted-nose monkey, *Cercopithecus nictitans,* and to consider the question of the parasite's specific affinities. Besides being an addition to the very few reports of the occurrence of a tapeworm larva of this type in a primate, a unique feature of this case is that the parasite exhibits the characteristic form of *Coenurus serialis,* which is generally regarded as being specifically restricted to connective tissue.

Briefly, the history of the host is as follows: The monkey was a juvenile specimen less than two years old. When about nine months of age, it had been brought to Boston by a sailor from some port on the West African Coast and sold to a local animal dealer.

The animal was kept as a household pet for about five months by its first American owners who found it unsuited to their requirements and therefore presented it to Doctor T. D. Jones of the House of the Good Samaritan in Boston. For nearly six months the monkey was kept in the hospital where it found favor as a pet and was never made the subject of experimentation. It was very lively and had shown no signs of illness until three or four weeks prior to death when a slight torticollis was noticed. About this time also a swelling commenced at the back of the head with definite softening of the underlying structures. The animal became comatose about six hours before death. There were no convulsions and at no time signs of paralysis.

So far as our enquiries showed, during its last 13 months of life the monkey had had no opportunity for coming into even remote association with dogs or dog excreta. Consequently, it seems probable, but by no means certain, that the ingestion of onchospheres that gave rise to the coenurus occurred in Africa.

The necropsy was performed by Dr. Jones. No lesions relating to the parasite in the brain were recognized in the thorax, abdomen or other parts of the body at the time of necropsy but a number of discolored bodies from one to two centimeters in diameter were observed attached to the mesentery. These were suspected of being enlarged, probably tubercular, lymph nodes with caseous contents. Subsequent sectioning, however, showed that the bodies in question represented encapsulated parasites in process of degeneration and absorption. Although the mor-

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phology is very indistinct, the parasite that induced the formation of these peculiar bodies is quite conceivably a coenurus which, in an unfavorable location, has failed to proceed with normal development. Some atrophy of the muscles at the back of the occiput and back of the neck was noted. Upon reflecting the scalp, there was found to be no bone erosion but the posterior fontanelle was found gaping open as a result of separation of the parietal bones mesially. The exposure was immediately followed by the protrusion of a mass of various sized, translucent, white cysts. Of these, some of the smaller were either separate or spontaneously became detached by the rupture of fine pedicels which connect the main body of cysts to form a single ramifying colony. Due to the massiveness of the parasite, the underlying occipital lobes of the cerebrum were markedly compressed.

The brain, together with the large number of cysts that still remain attached, was removed from the skull and forthwith preserved in formalin. The parasitic nature of the cysts having been recognized, the material was passed on to the writer and is now deposited in the Helminthological Collection of the Museum of Comparative Zoölogy at Cambridge.

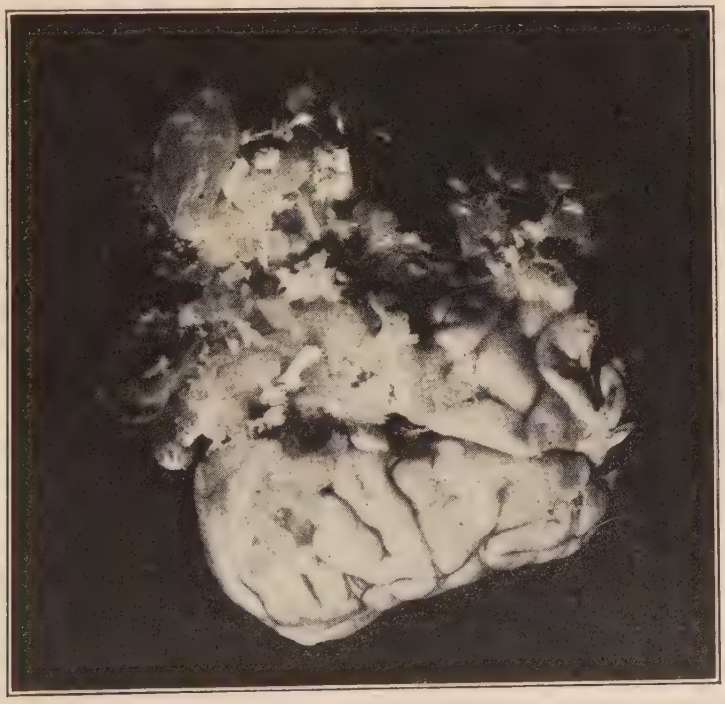

FIG. 1. Coenurus on the brain of a monkey. Parasite *in situ*. About one-half natural size.

Confirming the observation of the pathologist, the writer finds that the parasite presents the form of a series of translucent, irregularly-shaped cysts, varying in size from a few millimeters to nearly two centimeters. (See photographs, Figs. 1 and 2.) Some of the smaller vesicles

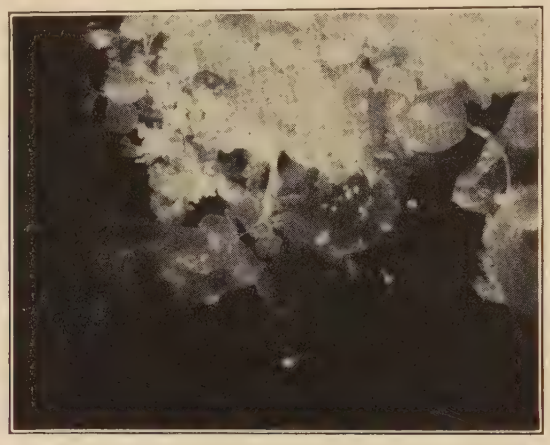

FIG. 2. Coenurus on the brain of a monkey. Actual size.

are free in the preserving liquid but the majority are united to each other forming a cluster attached by a stout peduncle to the falx cerebri. In no place has the parasite invaded the substance of the brain.

Examination of the larger cysts shows the coenurus nature of the parasite, with numerous (up to 40) "cysticercoids" attached in groups to the inner germinal layer of the walls of many of the larger cysts. Detached "cysticercoids" were never seen floating free in the liquid within the vesicle. Budding out from the wall of many of the cysts are a series of irregularly placed digitiform processes. These diverticula represent the early stages in the development of daughter cysts.

In the "cysticercoids," scoleces are found in various stages of formation. On immature scoleces, the hooks are relatively small and show various shapes, sometimes devoid of guards and often with mere rudiments of handles or roots. These are, in our opinion, incompletely developed rather than abnormally shaped hooks (Fig. 3, row C). Not a few abnormal scoleces, with only a single row of hooks or with supernumary suckers were also noted (*cf.* Henry, 1934).

The fully mature but still invaginated scolex after dissection shows the following characters: In unpressed preparations the diameter varies from 0.52 to 0.66 mm. The suckers measure from 0.19 to 0.24 mm. wide. The hooks, in the usual alternating rows, range in number from 26 to 30 with the majority of scoleces possessing two rows with 14 hooks each. The variation in the shape of the hooks, torn from the rostellum

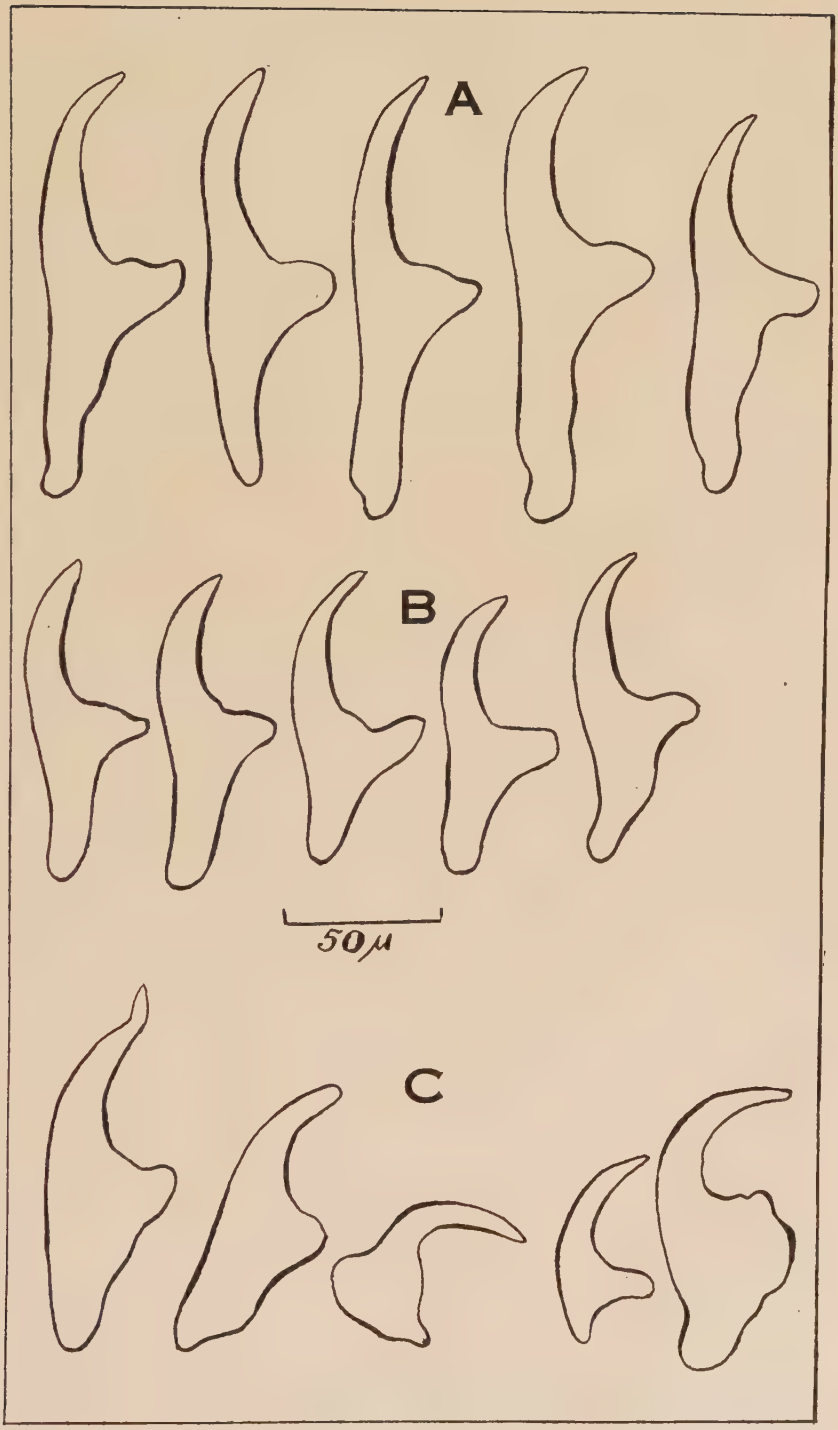

FIG. 3. Hooks from Coenurus from *Cerococebus nictitans* showing the variation in shape and size of the large and small hooks (rows A and B) and the range in shape of hooks on an immature or abnormal scolex (row C).

and mounted as far as practicable in a flat optical plane for drawing, is illustrated in figure 1. In length the large hooks measure from 120 μ to 145 μ; the small hooks measure from 68 μ to 100 μ.[1]

DISCUSSION ON SPECIFIC STATUS

Coenurus larvae are conventionally found in Ungulates (especially herbivores) and Rodents. However, there is a growing series of reports of their occurrence in primates, including at least half a dozen records in man. In taxonomic descriptions of these coenuri, the main emphasis is placed on the size of the scolex and more particularly on the size and form of the hooks. In addition to these morphological features, in most helminthological practise there is a great temptation to assign the parasite to a new species, should the host species be new. At the present time twelve separate species of *Coenurus* have been created. The strobilate adults of four of these presumptive species have been described after being secured by feeding the corresponding coenuri to dogs.

To the more critical student, the validity of several of these species of *Coenurus* is regarded as unproved, especially when the range in the size of the hooks, upon which the distinctness of the species has been proposed, overlaps or falls well within the range of variability of a species that has been more extensively studied. Even the strobilate adult *Multiceps,* which present many more structures for taxonomic discrimination than do the corresponding larvae, are subject to such variation in appearance that so competent a student of cestodes as Southwell (1930, p. 6) declares that it is practically impossible to distinguish one species from another. Yet, difficult as it undoubtedly is in actual practise to distinguish between the species, the experimental work of Baillet (1863) is generally cited as providing conclusive proof of the existence of at least a physiological difference between the two best known species in the genus, namely, *Multiceps multiceps* (Leake) and *M. serialis* (Gervais, 1847).[2] Allusion here is made to the presumed host specificity and histotropism exhibited by the coenurus which in the case of *M. multiceps* develops completely only in the brain and spinal cord of sheep, cattle and other herbivores, whereas the coenurus of *M. serialis* is a connective tissue parasite developing in subcutaneous tumors, between the muscles,

[1] My thanks are due to Dr. A. K. Mukerji for his careful work in making most of the above measurements and enumerations on 8 scoleces.

[2] For confirmation of this citation, the writer has studied Baillet's paper which is concerned primarily with the delineation of the life-cycle of the coenurus in rabbits and in proving that it is distinct from *Cystercus pisiformis.* Incidental to the main thesis, Baillet (p. 473) mentions his failure to infect sheep with onchospheres of rabbit origin and conversely to produce coenurus infections in rabbits with material originally derived from sheep. These failures may, perhaps, be attributed to other factors. It would seem that the question was of sufficient importance to warrant a repetition of the infection experiment accompanied by adequate controls.

on the peritoneum and occasionally on various abdominal viscera of rabbits and various other rodents. A difference in appearance of the coenurus is usually correlated with this difference in location and host; *multiceps* usually takes the form of a solitary bladder, spheroidal or simple in its contour, while *serialis* shows a disposition to external gemmation with the production of multiple, variably shaped and sized, daughter vesicles. This correlation, however, does not appear to be hard and fast, at least in rabbits (see Henry and Ciuca, 1914). Another noteworthy exception to the generalization is encountered in the goat in India where the habitat of the coenurus (referred by Hall (1919) to a new species, *M. gaigeri*) combines the central nervous system and the various connective tissue sites of the species *multiceps* and *serialis*. In commenting upon *M. multiceps,* Southwell (*loc. cit.*) suggests that it would not be surprising to find that the egg of this parasite finds conditions suitable for its development only in the brain of sheep, or, in the case of rabbits, in the connective tissue, whilst in the goat the conditions are equally suitable in both the previously mentioned sites.

Since the two common species of *Multiceps* are difficult or impossible to tell apart on morphological grounds, in the few instances where coenuri have been encountered in man it would appear that the specific determination has been arrived at on the basis of the gross form and/or the location of the cysts.

In what seems to have been the first well authenticated case of a coenurus in man (Brumpt, 1913) the location of the parasite in the brain of a Parisian appears to have been the sole basis for identifying it as *M. multiceps.*

In two subsequently recorded human cases of coenurus in France (Bonnal, Joyeux and Bosch, 1933, and Brumpt, Duvoir and Sainton, 1934) the parasite, lodged in subcutaneous tumors and exhibiting exogenous budding of daughter cysts, was identified with *M. serialis.* The erratic occurrence of these species in man in France must be considered as not unlikely because in this country the respective infections in sheep and in rabbits are quite prevalent. On the other hand, considerably less is known of the occurrence of a species of *Multiceps* in the fauna of equatorial Africa and the specific diagnosis of *serialis* or *multiceps* cannot be so readily attached to a parasite that fortuitously happens to have infected a man in that region. There are two records of coenurus in African natives. In one, where a unilocular cyst had been excised from the intercostal muscles of a North Nigerian, Turner and Leiper (1919) referred the parasite to the species *glomeratus* Railliet and Henry, 1915, which had been originally described from a gerbille of Tunis. The validity of *C. glomeratus* is rather dubious, for it was based on the size

and shape of hooks that are apparently imperfect or incompletely developed.

The second specimen, a simple cyst, was taken by Taramelli and Dubois from the arm of a native in the eastern part of the Belgian Congo. Baylis (1932) after examining the material found no warrant to attribute the parasite to any of the species previously recorded from man. This was not because it was considered necessarily a new species but on account of the difficulty of using the character of hooks for differentiating between the species already described. Summing up the situation, Baylis expressed the opinion that "it seems useless either to attempt to assign the human coenurus of Taramelli and Dubois to any known species, or to give it a new name which would be likely merely to burden the literature with yet another synonym."

From the lower primates, infections with coenuri have been recorded only three times. Here again the problem of determining the species involved is complicated by the probability of the parasite having established itself in the host in tropical regions where practically nothing is known concerning the occurrence of *Multiceps* in the autochthonous fauna.

Railliet and Marullaz (1919) reported a coenurus lodged in the perineum of a laboratory monkey, *Silenus* (*Macacus*) *sinicus*. The parasite apparently exhibited the *serialis* form and was described as a new species, *C. ramosus*.

Scott (1926) records multiple coenurus tumors in two baboons, *Theropithecus gelada,* that died in the London zoo. In one animal immense tumors were found in the breast, and invading the mediastinum, pericardium and thymus. In the pleural cavity of the second baboon, three cysts were found, one of which had penetrated an intercostal space into the vertebral canal, gradually compressing the spinal cord and giving rise to paralysis. The morphology of these parasites is not described and the species is not indicated.

In 1927, Schwartz published a very comprehensive account of a coenurus found in a breast tumor of a baboon, *Theropithecus obscurus* (synonym of *T. gelada*) that was destroyed in the Zoological Park at Washington, D. C. The parasite is probably specifically the same as those recorded in London by Scott. Schwartz described it very completely both in the coenurus stage and in the adult strobilated stage secured after the experimental infection of dogs. Its morphology was found to correspond closely with Hall's description of *M. serialis* and Schwartz was able to detect no constant differences between it and specimens of *M. serialis*. However, because he failed to establish infections in five rabbits and two lambs after administering onchospheres in

gravid segments to these laboratory animals, Schwartz came to the conclusion that the parasite was biologically distinct from *M. serialis* and he created the new variety, *M. serialis theropitheci,* to indicate the apparent physiological difference.

The parasite with which we have been concerned in this paper, coincides in all morphological features with Schwartz's comprehensive description and illustrations of the coenurus from *Theropithecus gelada.* However our case represents what we believe to be the first record of the occurrence of a coenurus of the *C. serialis* type in the *brain.* The conditions, therefore, are antithetic to the occurrence of the *M. multiceps* type of coenurus in the connective tissues, such as we have noted above in the human cases from Africa.

We have already outlined some of the anomalies that complicate the question of the unity or duality of the species *M. multiceps* and *M. serialis* and, identifying ourselves with Baylis's view on this subject, we believe that no useful purpose is served by giving a specific name to the parasite described in this paper. The entire taxonomic problem must be regarded as unsettled until extensive reciprocal infections with parasites from different hosts have been carried out.

SUMMARY

A natural infection with a species of coenurus is recorded from the brain of *Cercopithecus nictitans.* Morphologically the ramifying larva corresponds with *Coenurus serialis* (Gervais) which in rodents is a parasite of connective tissue. The present material may represent new evidence challenging the duality of the morphologically indistinguishable species *M. multiceps* (Leake) and *M. serialis* (Gervais). It suggests the desirability for a repetition of the cross infection experiments of Baillet (1863) upon the results of which the distinctness of the two species has generally been accepted.

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THE NATURAL HABITAT AND DISTRIBUTION OF *HARTMANNELLA CASTELLANII* (DOUGLAS), A REPORTED CONTAMINANT OF BACTERIAL CULTURES*

REDGINAL HEWITT

Castellani (1930) described an amoeba which was found by him in a mixed culture of *Cryptococcus pararoseus* and bacteria. He published a series of notes concerning the nature of the organism as it appeared in his cultures. A short time later, Douglas (1930) further described the organism and placed it in genus *Hartmannella* Alexeieff, basing his classification upon the character of the nucleus and type of cyst formed. He proposed the name *Hartmannella castellanii,* differentiating it from other species of *Hartmannella* in that, (1) it was strictly associated with certain fungi and bacteria, and growth did not take place in the absence of such organisms, (2) it produced an enzyme which liquified coagulated serum and gelatin, (3) it produced long, filamentous micropseudopodia previous to encysment. Van Rooyen (1932) published a rather extensive account of the reaction of this amoeba to various types of bacteria. Recently an amoeba resembling *Hartmannella castellanii* has been described by Shinn and Hadley (1936) as a contaminant on cultures of Friedlander's bacillus (*Bacterium pneumoniae*). They reviewed the characteristics of the organism, and their description indicates that it is probably *Hartmannella castellanii* or a variant of that species.

The purpose of the present paper is to record a further isolation of this interesting amoeba as a contaminant of bacterial cultures, and to present a brief account of its habitat and distribution, along with a review of its cultural characteristics. The fact that the organism has been isolated several times under similar conditions in widely different regions indicates a wide distribution, and its occurrence as a contaminant of bacterial cultures would seem to warrant a detailed study of its habits.

Morphology. The dimensions of this amoeba vary from twelve to thirty micra in diameter, with an average size of twenty-five micra. The nucleus varies from three to four micra in diameter, and contains a distinct central endosome (Fig. 1). Small granules of chromatin may generally be seen in the clear zone between the endosome and the nuclear membrane, but these are often difficult to make out in unstained preparations. There is generally one principal pseudopod, with occasional sec-

* The writer wishes to express his appreciation to Doctor R. D. Manwell, Department of Zoology, Syracuse University, and to Doctor Robert W. Hegner, Department of Protozoology of the Johns Hopkins University, School of Hygiene and Public Health, for helpful suggestions and criticisms.

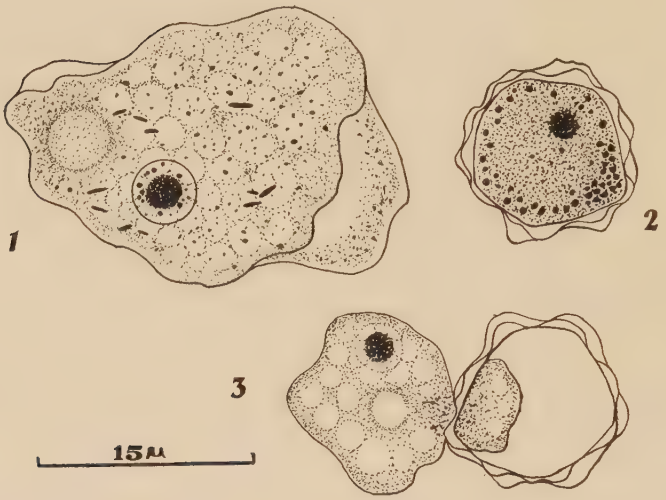
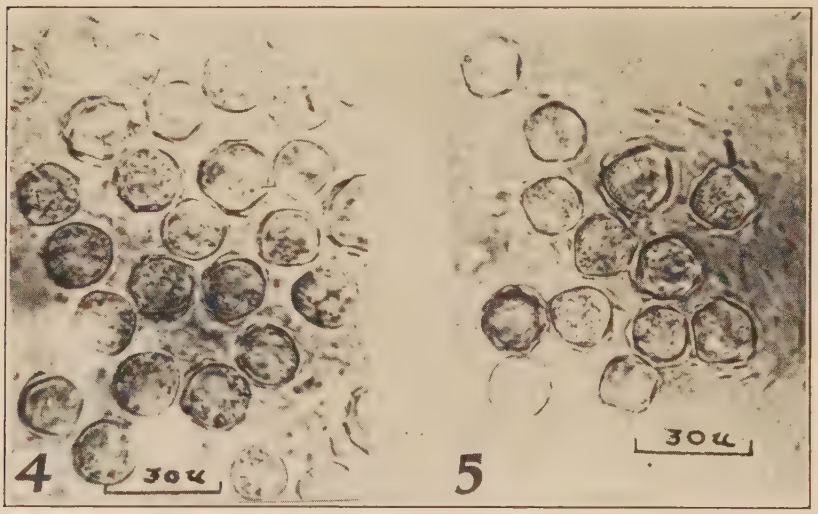


FIG. 1. Trophozoite of *Hartmannella castellanii*, showing the nucleus with its distinct endosome and scattered peripheral chromatin granules. Several bacteria have been ingested. (Drawn from a camera lucida sketch.)

FIG. 2. Mature cyst. The distinct large granules are of unknown function. (Drawn from a camera lucida sketch.)

FIG. 3. Germinating cyst. (Drawn from microphotograph.)

ondary pseudopodia. There may be one or two contractile vacuoles. Cysts formed by this species generally have two outer wrinkled walls and a smooth, roundish inner wall (Fig. 2, 3, 4 and 5). They vary in size from ten to fourteen micra in diameter, the average cyst measuring



FIGS. 4 & 5. Microphotographs of cysts taken with the oil immersion lens. Bacteria may be seen around the cysts. ×1000.

twelve micra. There is no distinct pore in the cyst wall and generally only one nucleus is present.

Cultural characteristics. In the present case the amoeba was found as a contaminant on mannite agar cultures of *Rhizobium,* the root nodule bacterium. Petri-dish dilutions were made from a sample of soil which surrounded the roots of white clover (*Trifolium repens*). Upon examination two weeks later several raised, warty, yellowish colonies were noted in addition to a few watery colonies of the root nodule bacterium. These warty colonies were found to contain a great number of angular cysts, along with a yeast-like fungus and a bacterium identified as *Flavobacterium trifolium* (Bergey *et al.*). The angular cysts gave rise to amoebae when transferred to a fresh medium.

As freshly transferred, the mixed colony of fungus, bacterium, and amoeba appears as a thin, watery growth which spreads rapidly over the surface of the plate or slant. Amoebae in the vegetative stage can be found in this growth in great numbers, feeding upon the fungi and bacteria. Within forty-eight hours the colonies begin to develop wrinkles and folds, and soon become hard and warty. Such colonies can be lifted *en masse* from the surface of the agar, much the same as a colony of *Actinomyces.* These hardened colonies contain cysts of the amoeba and very few vegetative forms can be found. At the end of two weeks no vegetative forms are present. The optimum growth temperature is 27° C.

The cysts were found to be very resistant to drying. A number of cysts were taken from a four-month-old agar culture, transferred to a glass slide, and placed in an incubator at a temperature of 37° C. At the end of three days some of the cysts were transferred to a fresh agar slant and many of them germinated within three hours. Cysts transferred to fresh agar slants at the end of six days showed 20 per cent germination within a day.

Habitat. Since the original isolation of the amoeba, it has been cultured from several different sources. The bacterial slime on the under surface of damp logs in wet weather has been found to contain considerable numbers of the vegetative stage of this organism. When transferred to agar slants, a typical colony was formed, containing bacteria and fungi (*Blastocystis* or *Fusarium*). A salient point here is that no amoebae were ever successfully cultured in the absence of fungi or bacteria.

Agar plates exposed to air within the laboratory never developed colonies of the amoeba, but they were isolated from flower pots and from dust deposits.

The normal habitat, then, appears to be quite varied. The organism occurs most frequently in the soil, probably in an encysted condition

during dry weather. The fact that it can be isolated so easily indicates that it is a very common form. The resistance of the cysts to drying accounts for its being carried from one place to another. Although it has never been isolated from the air, it may occur there in an encysted state when there is sufficient draft to carry the cysts about.

Discussion. The organism just described seems to be the same as that isolated by Castellani. The pictures of cysts which were included in his publications agree precisely with the photomicrographs included in this paper. Both Castellani and Douglas noted that previous to encystment the amoebae sometimes showed long, filamentous pseudopodia. These pseudopodia are probably not very common and may be the result of a specialized environment rather than the rule. The writer has never seen them. In all other respects, however, the results are in accord with the original observations of Castellani and Douglas.

Van Rooyen's extensive study of the reaction of *Hartmannella castellanii* to over fifty different bacteria brought out some interesting facts. His purpose was to discover the nature of the "clearing" effect noted by Castellani in cultures of the amoebae and bacteria. In his conclusions he states that it was impossible to group those bacteria upon which the amoeba feeds on taxonomic grounds. In other words, the clearing reaction cannot be restricted to any particular bacteriological group. He further concluded that the clearing of yeasts and bacterial cultures by this amoeba was simply the result of the growth of the amoebae and the ingestion by them of the fungi and bacteria. His investigations included pathogenic bacteria as well as non-pathogens.

The exact relationship between the amoeba and fungi in nature is not quite clear. As far as laboratory cultivation is concerned some fungal or bacterial growth must be present in order that the amoebae survive. This relationship may be carried over from the natural habitat, or it may be entirely due to chance that fungi, bacteria and amoebae have been isolated together. It has been demonstrated, however, by both Castellani and the writer that the amoebae do ingest fungi. In a fresh culture very few amoebae can be found which do not contain ingested fungi. Van Rooyen found that some bacteria could be demonstrated within the cytoplasm of the amoebae, and others could not. At any rate, all of the places from which the organism has thus far been isolated were rich in fungal and bacterial growth, and until the amoeba is found in a free-living state, free from these plants, it must be assumed that its existence is dependent upon them.

SUMMARY

1. The further occurrence of *Hartmannella castellanii* as a contaminant of bacterial cultures is recorded. Isolation was made from a

mannite agar Petri-plate culture of root nodule bacteria, in which several mixed colonies of *Flavobacterium trifolium* and the amoeba were found, separate from the root nodule bacteria.

2. This amoeba was further isolated from soil and from bacterial slime on the under surface of damp logs.

3. Fresh cultures of the amoeba in the presence of bacteria and fungi appear as thin watery growths, containing only vegetative stages. Cultures more than 48 hours old develop wrinkles and folds, later becoming hard and warty. Such cultures are nearly entirely composed of cysts.

4. The cysts are very resistant to drying, some remaining viable on a glass slide at a temperature of 37° C. for 6 days. This resistance of cysts to drying might well account for the wide distribution of the organism.

5. The fact that the organism has never been isolated in a true free-living state, and grows only in the presence of fungi and bacteria seems to indicate that it is dependent upon such plants for its existence.

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THE TICK PARASITE *IXODIPHAGUS TEXANUS* IN NYMPHS AND LARVAE OF *HAEMAPHYSALIS LEPORIS-PALUSTRIS* IN MINNESOTA*

CARL L. LARSON

The rôle of ticks as vectors in certain diseases has created a widespread interest in the discovery and application of factors leading toward control of ticks. The use of insect parasites as a means of control has occupied a prominent place in studies on the problem. Attempts to establish tick-parasite colonies have been reported by Cooley and Kohls (1934), Lounsbury (1908), and Larrousse, King and Wolbach (1928).

The insect parasites of ticks are minute chalcidoids belonging to the family Encyrtidae. Howard (1907) reported the occurrence of *Ixodiphagus texanus* in nymphs of *Haemaphysalis leporis-palustris* taken from a cottontail rabbit in Jackson County, Texas. Howard (1908) created a new genus and species for another parasite, *Hunterellus hookeri,* recovered from nymphs of *Rhipicephalus sanguineus* taken from a dog at Corpus Christi, Texas. Buysson (1912) reported a species, *Ixodiphagus caucurtei,* taken from nymphs of *Ixodes ricinus* occurring on deer in France.

Hunterellus hookeri has been reported over a widespread area in a great number of hosts. Specimens have been taken from nymphs of ticks belonging to five genera: *Ixodes, Haemaphysalis, Rhipicephalus, Dermacentor,* and *Hyalomma.* Recovery has been accomplished in Texas, California, South Carolina, Florida, Montana, Portugese East Africa, Brazil, Porto Rico, Cuba, Mexico, South India, Indo-China, Natal, the Transvaal, Pretoria district of South Africa, and Nigeria, West Africa. *H. hookeri* has a wider distribution in nature than does *I. texanus.* Since the original description only two other isolations of *I. texanus* have been made. One of these isolations was made in 1914 by Bishopp (1934) from nymphs of *Haemaphysalis leporis-palustris* found on a chaparral cock (*Geococyx californicus*) at Reagan Wells, Texas. Cooley (1934) reported another instance of natural occurrence when the parasites were discovered in nymphs of *Ixodes hexagonus* var. *cookei* on a woodchuck in Mayfield, Idaho, in 1932. The writer, at that time with the Public Health Service, assisted in the collections that resulted in this latter isolation.

It has been observed that these parasites occur naturally in a recognizable stage in only the engorged nymphal ticks. A report by Cooley

* From the Department of Bacteriology and Immunology, University of Minnesota, and the Bureau of Biological Survey, U. S. Department of Agriculture.

(1930) of a chalcid parasite attacking adult ticks has proved groundless. Wood (1911) states that fertilized females, when occupied in the act of oviposition, pay no attention to larval ticks. Cooley and Kohls (1928), however, have observed that eggs of *H. hookeri* are readily deposited in larval ticks when engorged larvae alone in large numbers are offered the females. Under these conditions, no signs of parasitism are observed during the subsequent changes in the tick, and the parasites begin to develop after the tick assumes the state of an engorged nymph. This phenomenon has been termed "latency" and appears to play an important rôle in the bionomics of this parasite. Recovery of parasites from naturally occurring larval ticks has not been accomplished previous to this report so far as can be determined from the literature.

The study of tick parasites in Minnesota here reported has been developed as a part of the wild animal disease investigations being conducted jointly by Dr. R. G. Green and Dr. J. E. Shillinger. During 1934 the tick-parasite, *Ixodiphagus texanus,* was recovered in Minnesota from immature rabbit ticks taken from four different animals. The first group of parasites originated from a cottontail rabbit shot on August 8, 1934, on the Lake Alexander Area, Morrison County, Minnesota. About 50 engorged nymphs were taken from this cottontail to be reared for experimental study. On September 8 it was noted that two of the nymphs presented an appearance typical of parasitized ticks: they were brown in color and their ventral surface was definitely rounded. On September 18, three parasites, two females and one male, had emerged from one of the nymphs under observation. The parasites failed to emerge from the other nymph, although they were found present when the tick was dissected.

In a pooled sample of ticks from two hares taken on the Lake Alexander Area in Morrison County, Minnesota, during the latter part of the summer of 1934, a number of parasitized ticks were noted. Six nymphs from which parasites later emerged were also taken from a group of ticks on a ruffed grouse collected in Pine County, Minnesota, on September 7, 1934.

Of especial importance is the discovery of a number of parasitized larval ticks which showed definite external changes associated with parasitism. Studies indicating that a large number of seeds occurring in Morrison County are being destroyed in that stage by actively developing tick parasites belonging to the species *I. texanus* have been pursued. A total of 17 engorged nymphs and 3 engorged seeds were collected from a snowshoe hare on the Lake Alexander Area, Morrison County, Minnesota, on April 25, 1935. These were sent to the laboratory at the University of Minnesota where they were observed at room conditions. On the fifteenth of June, 2 of the nymphs were found to be parasitized and

three seeds appeared to be abnormal. As they showed the same appear- as do parasitized nymphs, they were isolated in order to determine what the cause of the change might be. Two parasites (*I. texanus*) emerged from one of the seeds and one from another on the twentieth of June. The parasites failed to emerge from the others, but two fully developed adults were present, as was proved when the shell of the seed was opened. Tick parasites were isolated from a large number of seeds taken from a series of hares collected throughout the tick season on the Lake Alexander Area, and appear to be present in large numbers during the major part of the season.

The significance of these findings are of importance in many respects. *Ixodiphagus texanus* has never been recovered in an area where the climatic conditions are so adverse. It will be noted that the previous recoveries have been made in Texas and Idaho where the factor of climatic resistance does not inhibit the activities of tick parasites to the same extent as would be the case in Minnesota. The discovery of active parasitism of larvae or seeds of the rabbit tick by this insect indicates that latency is not a factor in the ability of *I. texanus* to maintain itself in nature. This fact may account for the large colony observed in Morrison County, Minnesota, and the apparently widespread distribution throughout the state.

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A NEW SPECIES OF BLADDER FLUKE, *GORGODERINA TANNERI* (GORGODERIDAE: TREMATODA), FROM *RANA PRETIOSA**

O. Wilford Olsen

The number of species of *Gorgoderina* Looss, 1902, has increased during recent years until at the present time there are thirteen reported in the literature. In this paper is described one more species which was found in about eighty per cent of the *Rana pretiosa* B. and G. collected at Springville, Utah. In honor of Dr. Vasco M. Tanner who has been so prominently associated with the development of the zoology of the surrounding regions, the name *Gorgoderina tanneri* sp. nov. is proposed.

Gorgoderina tanneri sp. nov.

Specific diagnosis.—*Gorgoderina*: Body subcylindrical, length of sexually mature specimens 2.07–7.67 mm. with an average of 5.25 mm. Average width 0.458 mm. Specimen figured measured 7.676 mm. long and 0.517 mm. wide. Ratio of length to width 1 : 6–1 : 14.5, average 1 : 11.37. Cuticle without spines.

Oral sucker terminal, spineless, average size 0.276 mm. long by 0.258 mm. wide; varies between 0.207–0.345 mm. long by 0.189–0.311 wide. Acetabulum in anterior quarter of body, spineless, more than twice as large as oral sucker, average diameter 0.621 mm., varies from 0.448–0.804 mm. Average ratio of oral sucker to acetabulum is 1 : 2.25.

Oesophagus averages 0.152 mm. wide by 0.508 mm. long, oesophageal glands absent. Intestinal caeca extend to near posterior extremity of body.

Genital pore ventral and median, near oesophageal bifurcation. Testes intercaecal, tandem, in posterior half of body and usually separated from each other by a distance about equal to or greater than the length of either; anterior testis near equator of body, the distance separating it from ovary usually about equal to its length. Anterior testis averages 0.476 mm. long by 0.267 mm. wide; posterior 0.564 mm. long by 0.238 mm. wide; vasa efferentia not seen, vas deferens enters seminal vesicle on posterior side midway between dorsal and ventral extremities; seminal vesicle thin walled, elongated dorso-ventrally, prostate duct arises on anterior side as a thin-walled structure midway between dorsal and ventral ends of vesicle, extends anterio-ventrad, then bends dextrad where it is surrounded by a mass of small glandular cells, then bends directly ventrad forming a slender portion without glandular cells which opens into the anterior half of the shallow genital pore. Ovary in posterior portion of anterior half of body, shape not fixed, usually longer than wide, average size 0.285 mm. long by 0.232 mm. wide; oviduct arises from a central mass of large cells on dorsal surface of ovary, extends dorsad, then bends anteriad forming an elongated seminal receptacle which extends to anterior margin of ovary; remainder of oviduct elongated with course somewhat sinous; Laurer's canal originates near end of first third of oviduct, extends dextrad; Mehlis' gland large, located at union of common vitelline glands and oötype. Descending and ascending limbs of uterus of small bore, very sinuous, fill entire area of body caudad of vitellaria except that occupied by genital glands, anteriorly from ovary the ascending

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limb greatly increases in diameter, forms six to eight loops terminating in a short, narrow, thin-walled metraterm. Vitellaria consist of two lobated organs, each with from four to six, rarely three, lobes extending dorsad, generally one gland, usually the right, bears one to two lobes more than the other; vitelline ducts unite mesally to form yolk reservoir ventral to anterior region of oötype, common vitelline duct short.

Excretory bladder Y-shaped, arms very short, stem long and slender terminating ventral to anterior testis, viewed laterally it is sigmoid in shape with a median dorsal bend. Flame cell pattern 2 [(4+4+4+4)+(4+4+4+4)]. Eggs in preserved specimens 0.027–0.030 mm. long by 0.0195–0.0234 mm. wide, average 0.030 by 0.0222 mm.

Host.—*Rana pretiosa.*

Habitat.—Urinary bladder.

Locality.—Springville, Utah, U. S. A.

Type No. 9044 deposited in the United States National Museum Helminthological Collection. Cotypes in Helminthological Collection of the University of Minnesota and of the author.

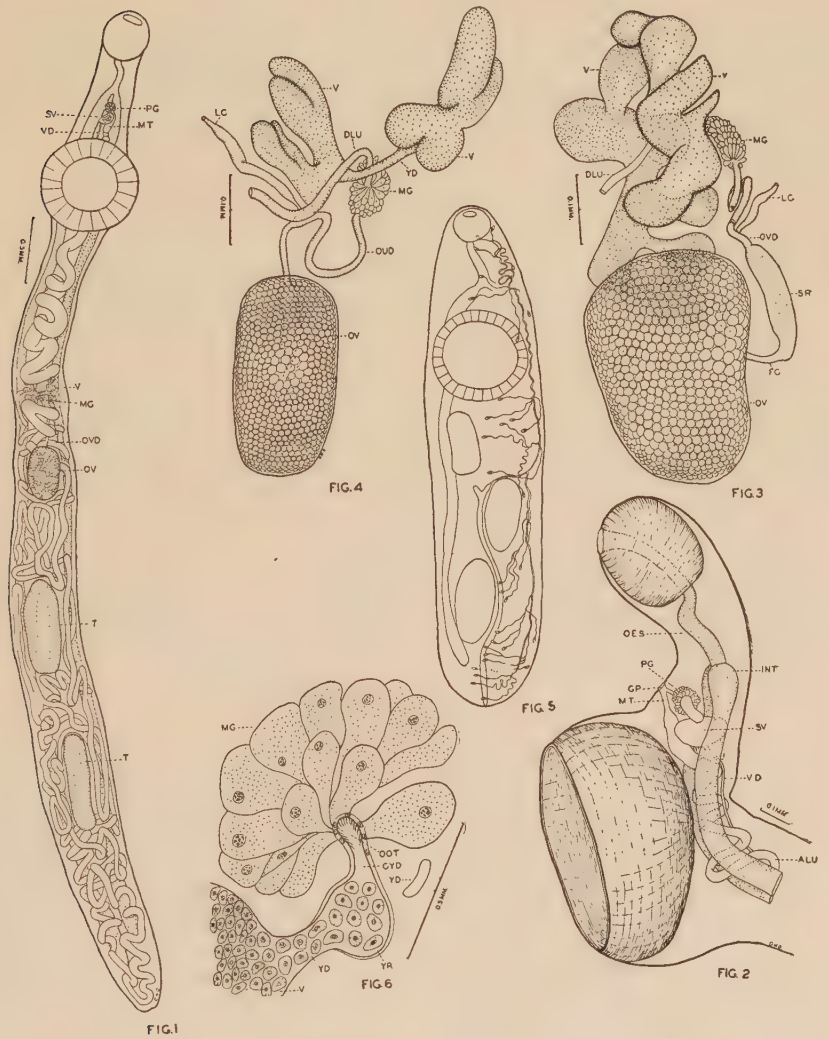
DIFFERENTIAL DIAGNOSIS

Gorgoderina tanneri together with *G. attenuata* Stafford, 1902, *G. capensis* Joyeux and Baer, 1934, and *G. aurora* Ingles, 1936, form a group distinguished from the other members of the genus in that the acetabulum exceeds the oral sucker in size by at least twice. *G. tanneri* is very closely related to *G. aurora* from which it may be distinguished, as well as from the other species, by the following characters: (1) regularly lobed vitellaria in which there occur four to six, rarely three, lobes each with a slender stem; (2) the greater distance separating the testes from each other and the anterior one from the ovary; (3) a more slender body, the average ratios of the length to the width being 1 : 11.37 and 1 : 9.5 for *G. tanneri* and *G. aurora,* respectively.

The remaining species of the genus have an acetabulum whose diameter is less than twice that of the oral sucker. Of these, *G. multilobata* Ingles and Langston, 1933, *G. translucida* (Stafford, 1902), *G. tenua* Rankin, 1937, and *G. bilobata* Rankin, 1937, resemble *G. tanneri* in that the vitellaria are lobed. It may be further differentiated from *G. multilobata* to which it shows closest affinity, by: (1) its sucker ratio; (2) fewer lobes in the vitellaria (4–6, *G. tanneri;* 7–9, *G. multilobata*);

Abbreviations

ALU	ascending limb of uterus.	OV	ovary.
CYD	common yolk duct.	OVD	oviduct.
DLU	descending limb of uterus.	PG	prostate gland.
FC	fertilization chamber.	SR	seminal receptacle.
GP	genital pore.	SV	seminal vesicle.
INT	intestine.	T	testis.
LC	Laurer's canal.	V	vitellaria.
MG	Mehlis' gland.	VD	vas deferens.
MT	metraterm.	YD	yolk duct.
OES	oesophagus.	YR	yolk reservoir.
OOT	öotype.		



Explanation of Plate

All drawings made with camera lucida except when otherwise stated.

Fig. 1. *Gorgoderina tanneri* n. sp., ventral view. The vitellaria are drawn with the lobes pushed laterally to show the structure.

Fig. 2. Lateral view of anterior portion of *G. tanneri* showing portion of male reproductive system.

Fig. 3. Left lateral view of female reproductive system, drawn from whole mount.

Fig. 4. Ventral view of female reproductive system. Drawn from whole mount.

Fig. 5. Free-hand drawing of heavily compressed young specimen of *G. tanneri* showing excretory system. Bladder supplied from sections of large adult specimen.

Fig. 6. Cross-section through Mehlis' gland showing relationship between glands, öotype, and vitellaria.

(3) absence of spines on suckers; and (4) absence of oesophageal glands. In addition to the sucker ratio, *G. tanneri* differs from *G. translucida* in having the posterior portion of the body cylindrical in form rather than flattened, and from *G. tenua* and *G. bilobata* in that it lacks oesophageal glands.

DISCUSSION

When Looss (1902: 851) erected the genus *Gorgoderina* to receive those species of *Gorgodera* with two testes, he did not include *Distomum vitellilobum* Olsson, 1876 from *Rana temporaria.* Since this species had been found but once and in view of its general agreement in structure with *Gorgodera cygnoides* (Zeder, 1800) on the one hand and its extreme rarity on the other, he was led to regard it as a variation of the latter. More recently Cort (1912: 153), Travassos (1922: 222), and Ingles and Langston (1933: 245) have included it in the genus *Gorgoderina.* In view of the continued lack of information relative to this form coupled with the fact that it was found in the stomach rather than in the urinary bladder, the organ inhabited by all the known species of this genus, that it was described as possessing a conspicuous pharynx, and that it otherwise shows general affinity toward *G. cygnoides,* it is not here included as a member of the genus *Gorgoderina.*

Key of Species of *Gorgoderina*

1. Acetabulum smaller than oral sucker ..... *G. parva* Travassos, 1920
   Acetabulum larger than oral sucker 2
2. Acetabulum twice or more than twice as large as oral sucker 3
   Acetabulum less than twice as large as oral sucker 6
3. Vitellaria distinctly regularly lobed, each with 4–6, rarely 3, follicles; testes more or less tandem, anterior testis separated from ovary by distance about equal to its length; testes rather widely separated . *G. tanneri* n. sp.
   Vitellaria compact or irregularly lobed but without follicles; testes close together, anterior one near ovary 4
4. Genital pore sinistral; testes tandem; ratio of oral sucker to acetabulum 1 : 2; ratio of body length to width 1 : 6.6 .... *G. capensis* Joyeux and Baer, 1934
   Genital pore median 5
5. Ovary heart- or kidney-shaped; smallest ratio of oral sucker to acetabulum 1 : 2.4; average ratio of body length to width 1 : 9.5; testes tandem. *G. aurora* Ingles, 1936
   Ovary oblong, elongated anterio-posteriorly; largest ratio of oral sucker to acetabulum 1 : 2.3; average ratio of body length to width greater than 1 : 9.5; testes obliquely placed ..... *G. attenuata* Stafford, 1902
6. Body with posterior region broad, flat, and transparent, thickness about one-third that of width ..... *G. translucida* (Stafford, 1902)
   Body subcylindrical throughout its length; posterior region thick and opaque 7
7. Oesophageal glands present 8
   Oesophageal glands absent 11
8. Vitellaria multilobed, divided into 7–9 grape-like follicles; suckers spinose. *G. multilobata* Ingles and Langston, 1933
   Vitellaria with much fewer or no lobes, suckers not spinose 9

9. Ovary larger than testes; vitellaria not lobate.
G. cryptorchis Travassos, 1924
Ovary not larger than testes; vitellaria lobate 10
10. Ascending limb of uterus forms 3–4 conspicuous and thick loops between vitellaria and acetabulum; oesophagus fairly long; eggs 35.6 × 22.2 μ; vitellaria bilobed ..... *G. bilobata* Rankin, 1937
Ascending limb of uterus without conspicuous loops between vitellaria and acetabulum; oesophagus extremely short; eggs 29.4 × 18 μ; one or both vitelline glands may be lobed but never with more than 4 lobes.
G. tenua Rankin, 1937
11. Anterior testis at level of ovary 12
Anterior testis removed caudad from ovary 13
12. Testes somewhat lobated; several loops of ascending limb of uterus occur between vitellaria and acetabulum which are well separated.
G. intermedia Holl, 1928
Testes with smooth contour; ovary, vitellaria, and anterior testis at level of caudal margin of acetabulum ..... *G. carli* Baer, 1930
13. Ovary and vitellaria far caudad from acetabulum; vitellaria irregularly lobulated ..... *G. simplex* Looss, 1902
Ovary and vitellaria at or near level of acetabulum; vitellaria rounded.
G. cedroi Travassos, 1924

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ECOLOGICAL STUDIES ON THE CERCARIAE IN *STAGNICOLA EMARGINATA ANGULATA* (SOWERBY) IN THE DOUGLAS LAKE REGION, MICHIGAN*

W. W. Cort, D. B. McMullen, and Sterling Brackett

INTRODUCTION

Very few investigations have been made of the ecological relations of larval trematodes in their molluscan intermediate hosts. One reason for this is that such studies require a sufficient knowledge of the cercariae found in any particular snail host or group of hosts so that the species present can be readily identified. The larval trematodes in the snails from the vicinity of the University of Michigan Biological Station have been studied since 1914, and in the last few years our knowledge has developed to the point that ecological investigations can be profitably undertaken. This is especially true for the cercariae of the common beach snail, *Stagnicola emarginata angulata* (Sowerby), which have been studied more extensively than those of any other species of mollusc in the region. In spite of this, six of the 21 different species of cercariae that have been found in this host are still undescribed. These forms, all of which are very rarely encountered, have been studied sufficiently so that they can be easily identified. Of the other 15 cercariae found in this host, the life cycles of 10 are known, and for the other five specific descriptions have been published. We decided, therefore, in the summer of 1935 to attack some of the ecological problems relating to the larval trematodes of this host. The collections of that summer yielded such interesting results that we extended the studies over the summer of 1936. In all, we have to present in the present paper the data on larval trematode infestation from 19 different collections of *Stagnicola emarginata angulata* from four separate areas making a total of 7,259 snails. These 19 collections are presented in Table 1 in 14 separate groups, since in four cases two or three collections brought in from one to seven days apart from the same area were combined into a single group for analysis. In these cases the grouping seemed justifiable, since there were no differences of any significance in the incidence figures for the cercariae in the collections joined into one group.

Perhaps the most striking thing in these collections was the frequency of multiple infestations. Therefore, in the first part of this paper a detailed analysis will be made of the occurrence of the different species of

* A contribution from the University of Michigan Biological Station and the Department of Helminthology, School of Hygiene and Public Health, The Johns Hopkins University.

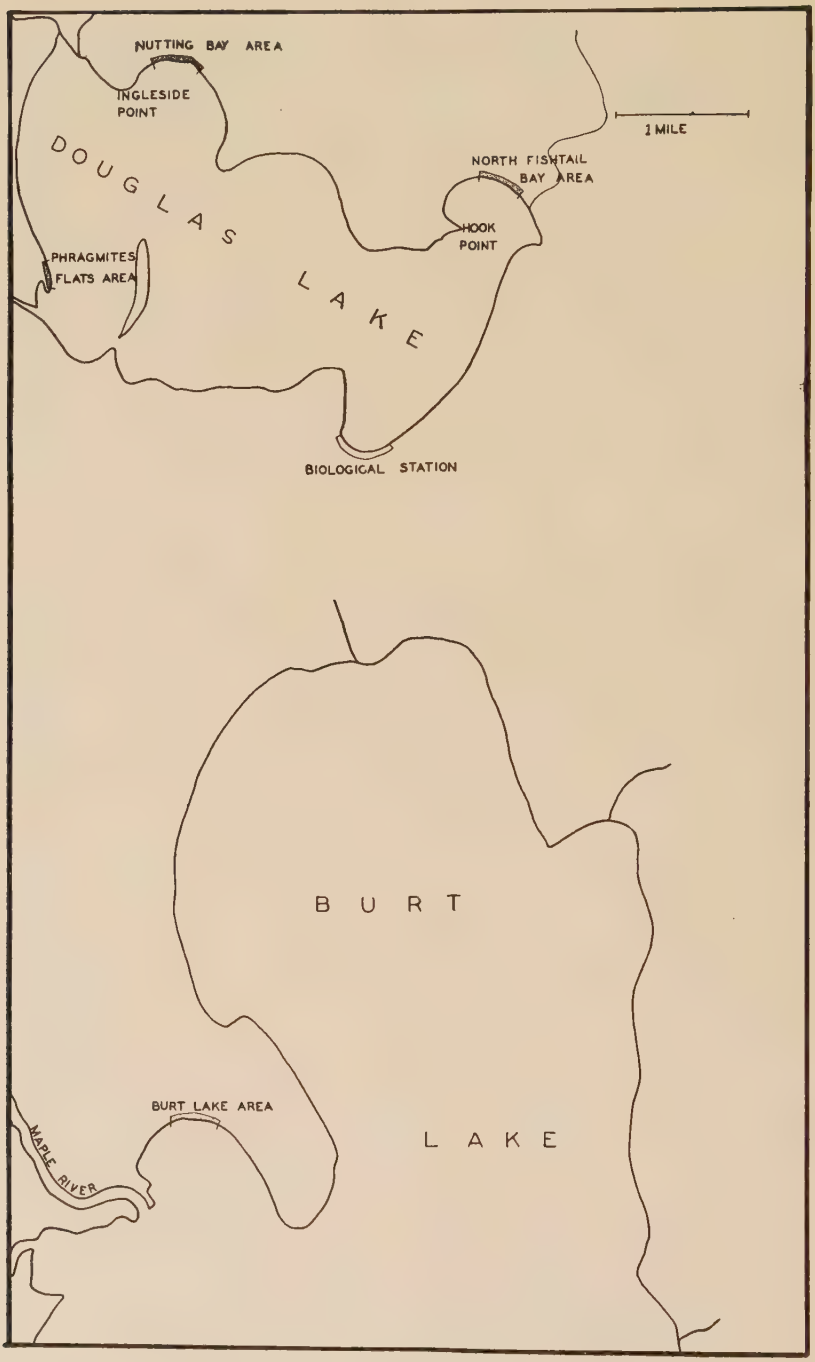
cercariae in multiple infestations. Also, the fact that from three of the areas collections were made during both the summers of 1935 and 1936 makes it possible to present data on the changes of infestation that were found in the same areas in different years. Further, collections were made from all four areas at different times during the summer of 1936. This makes it possible to present the changes that occurred in the incidence of the different species of cercariae in the same areas during the course of a summer season. From these changes some tentative suggestions can be made in regard to the seasonal cycle of infestation in this host of certain of the species of cercariae. The data obtained on the last two of these problems will be presented in the second part of this paper.

Before presenting the analysis we have been able to make on these three subjects, it will be necessary to describe and locate the areas from which the collections were made, to outline the methods used in handling the collections, and to give some discussion of what is known about the different species of cercariae found.

Descriptions of Areas from Which the Collections were Made

The four areas, from which the collections analyzed in this paper were made, are widely separated stretches of beach, three on Douglas Lake and one on Burt Lake (see map I). These collection areas had to be of such a size that the snails would be abundant enough to give the numbers needed for study. At the same time each area had to be as restricted as possible to insure that the snails over its whole extent had a somewhat similar exposure to infection with the miracidia of the larval trematodes present. Ideal conditions in this second respect would have necessitated the use of areas too small for the collection of the numbers of snails needed. That there was some unity in exposure to infection in each of the areas studied is indicated by the fact that collections made from different parts of the same area and on different days, when not separated by more than a week, showed approximately the same general picture of cercarial infestation.

As shown on map I the Burt Lake area is on the west side of Burt Lake a short distance east of the mouth of Maple River. The beach here has a southern exposure to several miles of open water. For this reason there is considerable wave action although it is protected from the prevailing northwest winds. Dense woods extend up to within six or eight feet of the shore line. There are no human habitations within a mile of the area. The collection area extends along this beach for about one-third of a mile. The eastern two-thirds of this beach is covered with cobblestones for a distance of about 10 yards from the shore line beyond which is a sandy shelf on which it is possible to collect snails for a distance of about 30 to 40 yards from the shore up to a depth of about two


NUTTING BAY AREA
INGLESIDE POINT
1 MILE
NORTH FISHTAIL BAY AREA
HOOK POINT
DOUGLAS LAKE
PHRAGMITES FLATS AREA
BIOLOGICAL STATION
BURT
LAKE
BURT LAKE AREA
MAPLE RIVER

MAP I

and one-half feet. In the western third the sandy beach comes clear up to the shore line. Collections here were made for about the same distance from the shore. Reeds are present on this beach especially toward the west, where it is bounded by the swamp surrounding the mouth of Maple River. The snails were rather sparsely scattered over this area at the time we made our collection, and it was often difficult to obtain the numbers needed for the individual collections, especially those made in August.

The North Fishtail Bay area is located at the northeast corner of Douglas Lake. The shore-line faces almost due south and is protected from the west by Hook Point. The collection area extends almost one-third of a mile along this beach. Just east of it is a small summer resort although there are no cottages on the beach where the collecting was done. This beach has a gradual slope and collections were made out for a distance of about 40 to 60 yards from the shore. Scattered patches or reeds extend out from the shore to distances varying from 10 to 30 yards. The snails were more numerous on the western half of this area. At the time of the June collection the snails were numerous, but in August the numbers needed were obtained with diffculty.

The Nutting Bay area is on the north shore of Douglas Lake facing south and protected on the west by Ingleside Point. There are several summer cottages along this shore and piers for swimming and attaching small boats extend quite a distance out into the water. The collection area is almost one-half a mile in length and the beach slopes out very gradually, so that collections could be made as far as about 75 yards from the shore. Scattered patches of reeds are present on this beach extending out about 60 yards from the shore. Snails were numerous but rather scattered over the area and were most frequently collected at a considerable distance from the shore.

The Phragmites Flats area is on the west shore of Douglas Lake with an eastern exposure. The collection area is about one-fifth of a mile in length. There are no habitations in the vicinity of the beach. The collections along this beach were made for a distance of only about 20 yards from the shore and the snails were numerous in shallow water. The snails were much more numerous on this beach than on any of the others studied. There is little vegetation on the beach. It faces a long stretch of open water but is somewhat protected by shallows further out, which are known as the Phragmites Flats.

For each individual collection an attempt was made to cover the whole of an area, so that the snails obtained would be from all parts of the area.

Methods of Studying Collections

When the collections of *S. emarginata angulata* from the areas described above were brought into the laboratory, the individual snails were

first isolated into half-pint milk bottles. The water in these bottles was later examined with a low power lens for cercariae that had escaped. The positives were then separated into groups and in some cases according to species from the preliminary identifications made from the appearance and behavior of the cercariae in the water. Very often multiple infestations could be spotted from this preliminary examination alone. This preliminary segregation made it possible to assign the different groups of cercariae to the one of the joint authors of this paper who was best qualified to make specific identifications. Then, as quickly as possible, all the snails of the collection were autopsied, the cercariae identified by microscopical examination, and the final records made of the species found. In all cases in this species of snail, the larval trematodes develop in the digestive gland. Only those infestations are listed in table 1 as immature in which the species of cercariae could not be identified. These, of course, are not included in the numbers used for each collection in computing the percentage of infestation with the different species of cercariae. A collection could usually be finished within two days after it was brought into the laboratory so that the number of snails that died during the period of examination was negligible.

Species of Cercariae Found

The larval trematodes in *S. emarginata angulata* in the region around the University of Michigan Biological Station have been studied since 1914. Our records show that 21 different species of cercariae have been found in this host. In the collections presented in this study (table 1) 17 of these species are present, viz., seven strigeids, two schistosomes, six plagiorchiids, one echinostome, and one monostome.

Of the strigeid species, by far the most common is the cercaria of *Diplostomum flexicaudum*. The adult of this species is found in the gull and the second intermediate hosts are certain species of fish (Van Haitsma, 1931 a). It can be seen from table 1 that the cercariae of this species occur in every one of the 14 groups with an incidence varying from 6.9 to 50.9 per cent. Throughout the whole Douglas Lake region, this species is very common and is present with a high incidence in practically every collection of *S. emarginata angulata*. It was chiefly its presence with such a high incidence that made the larval trematode infestations of this host so favorable for the study of multiple infestations. Next to the cercaria of *D. flexicaudum*, the most common strigeid species is the cercaria of *Cotylurus communis*. This species also has a fish-gull combination for its second intermediate and definitive hosts (Van Haitsma, 1930). It is seldom absent from collections of this species of snail from the region, but its incidence is almost always considerably less than that of the former species. Three other strigeids, *Cercaria laruei*,

TABLE 1.—*Incidence of Species of Cercariae in* Stagnicola emarginata angulata *from 14 groups of Snails Collected from Four Areas in Douglas Lake Region*

	Burt Lake Area						North Fishtail Bay Area			Nutting Bay Area			Phragmites Flats Area	
	I 7/23/35	II 8/21/35	III 6/23/36	IV 7/11/36	V 8/11/36	VI 8/20 and 23,'36	I 8/23 and 26, '35	II 6/29/36	III 8/17/36	I 8/18, 23, and 26, '35	II 6/26/36	III 8/30 and 31, '36	I 7/6/36	II 8/26/36
Total number of snails in groups	187	294	450	474	462	460	227	551	486	1,306	521	593	753	495
Number of immature (unidentified) positives	16	3	29	25	29	5	5	53	5	17	8	18	30	12
Total number minus immature positives*	171	291	421	449	433	455	222	498	481	1,289	513	575	723	483
Percentage of identified positives	95.9	93.8	45.8	71.7	87.3	87.0	89.6	38.2	87.7	62.5	27.1	41.6	35.4	67.9
Percentage incidence of species of cercariae:														
STRIGEIDS														
Cercaria of *Diplostomum flexicaudum*	40.9	42.6	10.9	19.2	32.3	31.9	50.9	19.7	42.2	41.0	11.5	17.4	6.9	20.9
Cercaria of *Cotylurus communis*	2.3	1.7	4.8	4.9	2.5	2.2	5.0	5.6	13.5	4.6	2.5	3.8	16.7	12.0
Cercaria laruei	0.6	1.4	1.4	0.2	2.1	2.4	1.8	0.2	4.0	0.8	0.4	1.9	0.1	2.1
Cercaria yogena	0.6	1.4	0.2	2.9	10.9	7.3	7.7	0	9.1	3.2	0	1.6	1.7	3.7
Cercaria dohema	2.9	0.3	1.0	1.8	6.9	4.6	0.5	0	0	0.4	0.2	1.0	0.1	0
Cercaria of *Cotylurus flabelliformis*	0.6	0	1.0	0.4	0	0	0	0	0	0	0	0	0.1	0.2
Holostome Z†	0	0	0	0	0.2	0	0	0	0	0	0	0	0	0
SCHISTOSOMES														
Cercaria stagnicolae	20.5	15.1	7.4	33.0	17.8	16.5	5.9	0.2	22.9	3.1	0	0.9	3.0	7.5
Cercaria of *Schistosomatium douthitti*	0	0	0	0	0	0	0	0	0	0	0	0	0	0.2
PLAGIORCHIIDS														
Cercaria of *Plagiorchis muris*	33.3	41.6	19.0	16.9	28.9	38.0	21.6	5.2	12.1	6.9	7.0	9.7	3.7	9.1
Cercaria of *Plagiorchis proximus*	0	0	0.2	0.2	0	0.7	2.7	7.4	7.1	5.2	5.3	6.8	4.0	20.3
Cercaria of *Plagiorchis microcanthus*	0	0.7	1.0	0.4	0.2	0.7	0	0.4	1.5	0.1	0.2	0	0	0
Cercaria of *Eustomos chelydrae*	0	0.3	0	0.2	0.7	0	0.5	0.6	0	0.2	0	0	0.4	0
Stylet G†	0	0	0	0	0	0.2	0	0	0.4	0	0	0	0	0.8
Stylet K†	0	0.7	0	0	0.2	0	0	0	0	0	0	0	0	0.2
ECHINOSTOMES														
Cercaria of *Echinostomum revolutum*	2.3	0.7	0.7	0.2	0.9	1.5	4.5	0.4	0.2	1.6	0	0	0.1	0.6
MONOSTOMES														
Cercaria of *Notocotylus urbanensis*	1.8	0	0.7	0	0.2	0.2	0	0	0	0	0.2	0	0.1	0.1

* The incidence percentages are based on this figure.
† Undescribed species for which laboratory designation is used.

C. yogena, and *C. dohema,* are present in most of the collections of this series but usually with rather low incidence (for descriptions see Cort and Brooks, 1928, and Cort and Brackett, 1937). The life cycles of these three species are not known. Somewhat scarcer than any of the last three species is the cercaria of *Cotylurus flabelliformis.* The adult in this case is a parasite of the duck and certain species of snails serve as the second intermediate host (Van Haitsma, 1931 b). One further strigeid cercaria, which is as yet undescribed, is listed in table 1 under its laboratory designation, Holostome Z. It was found in only one snail from a collection from the Burt Lake area.

Of the schistosomes, *Cercaria stagnicolae,* described by Talbot (1936), is common in the collections. It is the most important dermatitis-producing schistosome cercaria of the area and appears to be the chief cause of the dermatitis on the bathing beaches (Cort, 1936). Its life history is not known but the adult probably lives in birds. The cercaria of *Schistosomatium douthitti,* which is commonly found in the region in other snail hosts (Cort, 1936), is present in one snail in one of the collections from the Phragmites Flats area. This form is also a dermatitis-producing schistosome cercaria and its adult lives in small mammals (Price, 1931).

Next to the strigeids, the plagiorchiids have the best representation in our series (table 1). By far the commonest of them is the cercaria of *Plagiorchis muris,* the life cycle of which was worked out for the Douglas Lake region by McMullen (1937). Next to the cercaria of *D. flexicaudum* this species is the most common cercaria of the region in *S. emarginata angulata.* It has a wide variety of definitive hosts both birds and mammals. The cercaria of *Plagiorchis proximus* (see also McMullen, 1937) is also quite common in most of the collections. Its definitive host is the muskrat. The cercariae of a bat plagiorchiid, *P. microcanthus,* and of a turtle form, *Eustomos chelydrae,* are present in a considerable proportion of the collections, but with a very low incidence. Their life cycles were also worked out in this region by McMullen (1935 and 1937). Two other plagiorchiids listed in table 1 under their laboratory designations, Stylet G and Stylet K, since they have not yet been described, are present in a few snails in some of the collections. Their life cycles are not known.

The only echinostome present is the cercaria of *Echinostomum revolutum* (see Johnson, 1920) and the only monostome, the cercaria of *Notocotylus urbanensis* (see Luttermoser, 1935). These species are found also in other intermediate hosts in the region.

Such a series of different species of cercariae in this one host, several of them very common, produced in these collections a large number of multiple infestations (table 2) and made it possible to get some idea of

whether the various combinations occurred as frequently as would be expected by chance (see part I).

Part I. Multiple Infestations of Cercariae

Historical

Since the statement of de Filippi (1857) that he frequently found *Cercaria cristata* associated with other species of cercariae in the same individual snails, occasional reports of infestations of single snails with two or even three different species of cercariae have appeared in the extensive literature on larval trematodes. Two authors, Sewell (1922) and Dubois (1929), in their monographs on larval trematodes commented on the rarity of such infestations in their collections. Wesenburg-Lund (1934, p. 149), on the other hand, indicated by the following statement that double infestations were common in his material: "For my own part I have never opened heavily infected material from a pond without finding a percentage of doubly infected specimens. The double infections consist either of two species of the same group, *e.g.*, two sorts of sporocysts of Xiphidiocercariae, developing quite different Cercariae or two species belonging to two quite different groups, in most cases Echinostomes together with Xiphidiocercariae or Monostomes." Further discussion by this author, however, indicates that he was considering as double infestations both the cases in which two different species of cercariae develop in the same snail and those in which the metacercariae of one species occur with the cercariae of another. These are obviously two entirely different relationships. In the first case, the same snail is serving at the same time as the intermediate host of two different species of digenetic trematodes, while in the other the snail is the intermediate host for one species and the second intermediate host for the other. Wherever species of trematodes are present that utilize snails for second intermediate hosts, such as many of the echinostomes and certain of the strigeids, a very large proportion of the snails in a given collection, whether infested or not with cercariae, may contain the metacercaria stages of these species. In the present discussion only those cases are considered in which the snails harbor at the same time the cercariae of different species. This means that the miracidia of two or more different species of digenetic trematodes have penetrated into the same snail and that development has proceeded to the cercaria stage.

Sewell and Dubois appear to be the only authors who have made any analysis of the occurrence in snails of multiple infestations of cercariae. Sewell (l.c., p. 19) reported the finding of 18 cases of double infestations from the examinations of nearly 4,000 snails. He concluded that their rarity indicated "that there is some mechanism at work which renders such double infections difficult of accomplishment." He gave in the fol-

lowing statement his idea of this mechanism: "Unless the miracidia enter the body of the snail simultaneously or nearly so, the effect of the growth and development of the first invader will be to damage the liver or gonad and produce physiological changes which may destroy the chemotactic stimulus that is normally present, and in consequence few, if any, miracidia would attempt to enter the already infected host; or on the other hand it may be that the damage and destruction of the gonad may be the causative agent, preventing the second invader from undergoing its normal development."

Dubois (*l.c.*, p. 19) found 29 instances of double and three of triple infestations in the total of 691 positives in the 1,190 molluscs which he dissected in Switzerland over a period of four years. He also emphasized the rarity of such infestations in his material and stated that it proved the reality of the phenomenon of immunity following the penetration of the first miracidium (". . . prouve la realite du phenomene de l'immunite a la suite de la penetration du premier miracidium"). He inclined to the view suggested by Sewell that the entrance of the miracidium of one species into a snail brings about a change which destroys or neutralizes its chemical attraction for other species of miracidia. According to this view, multiple infestations only occur when two or more species of miracidia penetrate a snail simultaneously, or within a very short period of time.

Neither Sewell or Dubois considered the mathematical probabilities of the occurrence of multiple infestations in any of their collections of snails. Also, they did not present their data in such a form that the probabilities for individual collections can be tested. Both of these authors were dealing with a large number of collections of a variety of different molluscs from a number of different locations. Also, only in a few cases did they find a high incidence of any species of cercariae in a particular snail host (see Sewell, 1922, table 1, p. 4, and Dubois, 1929, table 1, p. 12). It seems probable, therefore, that in most of their collections, at least, the incidence of the cercariae present was too low to produce multiple infestations. There is, therefore, no real evidence from their findings of the presence of an immunity or an antagonism preventing such infestations.

In this connection, it may be worth while to consider a purely hypothetical case. Let us imagine a collection of 500 snails of the same species from an area so limited that all would be expected to be equally exposed to infection with any miracidia that would be introduced. They are found to be infested with three species of larval trematodes as follows: Cercaria A, 10 per cent; Cercaria B, 5 per cent, and Cercaria C, 2 per cent. It is postulated that there is no immunity or antagonism to the production of multiple infestations between these three species of larval

trematodes. Mathematically the chance number of double infestations that would be expected between A and B in this hypothetical collection would equal $0.10 \times 0.05 \times 500 = 2.5$. The number of doubles of A and C that would be expected would be 1 and of B and C, 0.5. The number of triple infestations that would be expected by chance would be 0.05, or but one chance in 20 that one such combination would occur. This gives a general idea of the incidence of different species of larval trematodes necessary in a collection before any considerable number of multiple infestations would be expected even if the combinations occurred strictly by chance.

It seems evident, therefore, that in order to prove that there is any immunity or antagonism that prevents any two given species of larval trematodes from occurring in double infestations, it is necessary to demonstrate that the actual occurrence of such combinations is significantly less than would be expected by chance in the particular collection of snails examined. It also seems evident, that only occasionally would collections of snails be obtained in which the incidence of infestation of the different species of larval trematodes present would be such that multiple infestations would be expected to occur in any considerable numbers. In the collections of *S. emarginata angulata,* which are available for analysis in the present study, the incidence of the various species of cercariae is such that multiple infestations were found in large numbers. This has made it possible in the discussion which follows to make a much more extensive analysis of multiple infestations than has ever been done previously.

Number and Combinations of Multiple Infestation.

In table 2 are listed the multiple infestations found in the groups recorded in table 1. The total number of multiple infestations is 529 or 12.3 per cent of the 4,304 identifiable positives. Of these, 511 are double, 17 triple, and one quadruple. The number of snails with multiple infestations varies in the different groups from 1 to 113. The smallest numbers, as would be expected, are in the groups with the lowest incidence of infestation, that is, those having less than 50 per cent positives. In these the percentage of the positives that show multiple infestations is in all cases less than six. An example of how greatly an increase in the incidence of infestation can increase the number of multiple infestations is shown by a comparison of groups II and III of the North Fishtail Bay area (table 2). Here in collections of about the same size with an increase of incidence of positives from 38.2 to 87.7, there is an increase from 8 to 113 in the number of multiple infestations; that is, while the percentage of positives is only a little more than doubled the percentage of the positives with multiple infestations increased over six times. It is

TABLE 2.—*Records of multiple infestations in the collections of* Stagnicola emarginata angulata *listed in table 1*

	Burt Lake Area						North Fishtail Bay Area			Nutting Bay Area			Phragmites Flats Area		Total for all Areas
	I	II	III	IV	V	VI	I	II	III	I	II	III	I	II	
DOUBLE INFESTATIONS															
Strigeids with Strigeids															
Cer. *D. flexicaudum* + Cer. *C. communis*	1	4	2	2	2	4	5	6	19	21	1	2	2	11	82
Cer. *D. flexicaudum* + *Cer. laruei*	0	1	0	0	0	2	1	1	5	2	0	2	0	2	16
Cer. *D. flexicaudum* + *Cer. yogena*	1	1	0	0	13	7	5	0	7	10	0	2	0	2	48
Cer. *D. flexicaudum* + *Cer. dohema*	0	0	0	0	4	3	0	0	0	1	0	1	0	0	9
Cer. *C. communis* + *Cer. laruei*	0	0	0	0	0	0	0	0	2	0	0	1	0	0	3
Cer. *C. communis* + *Cer. yogena*	0	0	0	0	0	0	1	0	2	2	0	0	0	1	6
Cer. yogena + *Cer. dohema*	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
Cer. dohema + Holostome Z	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
Strigeids with Schistosomes															
Cer. *D. flexicaudum* + *Cer. stagnicolae*	8	20	3	28	11	5	8	1	42	12	0	1	6	6	151
Cer. *C. communis* + *Cer. stagnicolae*	2	0	2	6	0	2	0	0	10	1	0	0	4	3	30
Cer. laruei + *Cer. stagnicolae*	0	0	0	0	2	1	0	0	1	0	0	0	0	1	5
Cer. yogena + *Cer. stagnicolae*	0	0	0	0	3	2	1	0	2	1	0	0	0	2	11
Cer. dohema + *Cer. stagnicolae*	0	0	0	0	1	1	0	0	0	0	0	0	0	0	2
Cer. *C. flabelliformis* + *Cer. stagnicolae*	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
Strigeids with Plagiorchiids															
Cer. *D. flexicaudum* + Cer. *P. muris*	1	1	0	0	4	7	0	0	0	3	0	0	0	0	16
Cer. laruei + Cer. *P. muris*	0	1	0	0	0	0	0	0	1	0	0	0	0	0	2
Cer. yogena + Cer. *P. muris*	0	0	0	0	4	5	2	0	6	1	0	0	0	1	19
Cer. dohema + Cer. *P. muris*	0	0	0	0	1	1	0	0	0	0	0	0	0	0	2
Cer. laruei + Cer. *P. proximus*	0	0	0	0	0	0	0	0	1	0	0	0	0	1	2
Cer. yogena + Cer. *P. proximus*	0	0	0	0	0	0	0	0	1	1	0	0	0	0	2
Schistosomes with Plagiorchiids															
Cer. stagnicolae + Cer. *P. muris*	5	7	2	3	17	38	2	0	0	3	0	0	0	3	80
Cer. stagnicolae + Cer. *P. proximus*	0	0	0	0	0	1	0	0	6	1	0	0	1	7	16
Cer. stagnicolae + Cer. *P. microcanthus*	0	0	0	0	1	1	0	0	1	0	0	0	0	0	3
Cer. stangicolae + Stylet K	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Plagiorchiids with Plagiorchiids															
Cer. *P. muris* + Cer. *P. proximus*	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2
TRIPLE AND QUADRUPLE INFESTATIONS															
Cer. *D. flexicaudum* + *Cer. stagnicolae* + Cer. *P. muris*	0	1	0	0	0	2	0	0	0	0	0	0	0	0	3
Cer. *D. flexicaudum* + Cer. *C. communis* + *Cer. stagnicolae*	0	0	0	1	2	0	0	0	0	0	0	0	0	0	3
Cer. *D. flexicaudum* + *Cer. laruei* + *Cer. yogena*	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
Cer. *D. flexicaudum* + *Cer. yogena* + *Cer. stagnicolae*	0	0	0	0	0	1	0	0	1	0	0	0	0	0	2
Cer. *D. flexicaudum* + Cer. *C. communis* + *Cer. yogena*	0	0	0	0	0	0	0	0	2	0	0	0	0	1	3
Cer. *D. flexicaudum* + *Cer. laruei* + *Cer. stagnicolae*	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
Cer. *C. communis* + *Cer. yogena* + *Cer. stagnicolae*	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
Cer. laruei + *Cer. yogena* + *Cer. stagnicolae*	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
Cer. yogena + *Cer. stagnicolae* + Cer. *P. muris*	0	0	0	0	1	0	0	0	0	0	0	0	0	1	2
Cer. *D. flexicaudum* + Cer. *C. communis* + *Cer. yogena* + *Cer. stagnicolae*	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
Number of Double Infestations	18	35	10	39	64	81	25	8	106	59	1	9	13	43	511
Number of Triple and Quadruple Infestations	0	1	0	1	4	3	0	0	7	0	0	0	0	2	18
Total Number of Multiple Infestations	18	36	10	40	68	84	25	8	113	59	1	9	13	45	529
Percentage of Positives with Multiple Infestations	11.0	13.2	5.2	12.4	18.0	21.2	12.6	4.2	26.8	7.3	0.7	3.8	5.1	13.7	12.3

evident, therefore, that in these collections a considerable number of multiple infestations are present only when the incidence of positives is considerably over 50 per cent and at least two species of cercariae have a rather high incidence. The fact is, that except for *S. emarginata angulata,* there are only a few cases in which the numerous collections of the wide variety of different snails that we have examined for larval trematodes in the Douglas Lake region have had the type of infestations that would make any considerable number of multiple infestations possible.

In the whole series of 14 groups, the double infestations occur in 25 different combinations (see table 2). With 17 different species of cercariae present there would be theoretically 136 different combinations possible. It is obvious, that an important reason why the actual number of different combinations is only a fraction of those possible is that so many of the species of cercariae are of such infrequent occurrence. For example, Holostome Z is present in only one snail in the whole series and then in combination with *C. dohema.* Also, the cercaria of *Schistosomatium douthitti* only occurred once in a single infestation. There are a number of other cases also, where the chances of occurrence of certain combinations are practically ruled out by the low incidence of the two species. There are some cases, however, where combinations are not present at all, when if they occurred strictly by chance, they would have been expected rather frequently. A striking example of this is the combination of the cercaria of *D. flexicaudum* with that of *P. proximus.* Other combinations that are entirely absent that would have been expected at least several times according to chance are those of the cercaria of *C. communis,* with the cercariae of *P. muris* and *P. proximus* and the cercaria of *Echinostomum revolutum* with the cercaria of *Diplostomum flexicaudum.* The relation of this to the possibility of an immunity or antagonism in the forming of certain combinations will be discussed later in this section.

The chances of the occurrence of triple or quadruple infestations of cercariae in a collection of snails is of course very much less than for doubles. As shown in table 2, we actually found in our material 17 triple infestations in nine different combinations. It can be seen that these are present only in the groups with a high incidence of positives where there were several species of cercariae with a rather high incidence. This number of triple infestations is several times all those previously reported in the literature. The finding of four different species of cercariae, the cercaria of *D. flexicaudum,* the cercaria of *C. communis, C. yogena,* and *C. stagnicolae,* that had developed side by side in the digestive gland of the same snail in group III of the North Fishtail Bay area is perhaps of special interest. We have found no other cases of quadruple infestations reported in the literature. In this instance all four of the species were

fully mature and escaping from the snail. It is of interest that the three strigeids in this combination are also present in two snails in this group in triple infestations.

In our collection of *S. emarginata angulata* only five kinds of group combinations are present in the double infestations, viz., strigeid with strigeid, strigeid with schistosome, strigeid with plagiorchiid, schistosome with plagiorchiid, and plagiorchiid with plagiorchiid. The echinostome and monostome were not found at all in double infestations. A great variety of group combinations were reported in the comparatively small number of double infestations observed by Sewell and Dubois. Sixteen of Sewell's 18 cases of double infestations occurred in *Melanoides tuberculatus* and the other two, in *Indoplanorbis exustus*. The groups of cercariae represented in the infestations of these hosts in India are entirely different from those found by us in *S. emarginata angulata* in the Douglas Lake region. In Sewell's series of double infestations 16 were mature enough for species identification of the cercariae. In these, 12 different species of cercariae occurred in 14 different combinations. His Cercariae Indicae III and VII appear to be heterophyids. They were found together. They also both combined with Cercaria Indicae XIII, which is probably a clinostome, and with strigeids and plagiorchiids. He also had the following other group combinations: a megalurid with a strigeid and a plagiorchiid, a strigeid with a plagiorchiid, a monostome with a plagiorchiid, and a clinostome with a plagiorchiid. Dubois had a more limited series of group combinations (see l.c., p. 20), viz., plagiorchiid with plagiorchiid, plagiorchiid with strigeid, plagiorchiid with echinostome, plagiorchiid with monostome, and plagiorchiid with cercariaeum. Cort (1915) reported a combination of a plagiorchiid and a gymnocephalous cercaria, and Faust (1917) an echinostome and a strigeid. It seems very probable that there are other group combinations in double infestations reported in the extensive literature on larval trematodes that have not come to our attention. There is nothing in these reports on multiple infestations or in our own material to suggest immunity or antagonism between whole groups of cercariae in the formation of double infestations.

Do Double Infestations Occur Strictly by Chance?

As stated above it is possible to determine mathematically from the incidence of larval trematodes in a collection, whether double infestations occur as frequently as would be expected by chance. For example, the number of double infestations of the cercaria of *D. flexicaudum* and *C. stagnicolae* that would be expected by chance in group I of the Burt Lake area (see tables 1 and 2) would be the product of their incidence times the number of snails in the collection, or $0.409 \times 0.205 \times 171 = 14$. The

actual number of snails found in this group with this combination is eight. To find in any collection of a given species of snail, which harbored infestations of two or more species of larval trematodes, the number of double infestation which approximated the number expected by chance, would presuppose, even in the complete absence of any immunity or antagonism, a rather ideal set of conditions. In the first place it would be necessary to find and identify the cercariae in all the double infestations present. It seems probable that even if all the cercariae are mature a few double infestations would be missed by any method of examination that would be practicable. Also, most cases of double infestations in which one of the species was immature would be missed except where one species developed in sporocysts and the other in rediae. More important still, is the fact that the mathematical determination of the chance occurrence of double infestations assumes that all of the snails in a given collection were equally exposed to infection with the miracidia of the different species of larval trematodes present. Such a situation can hardly be conceived except under experimental conditions, or in an extremely limited area in the field. It seems obvious, that in the rather extensive areas from which we made our collections equal exposure to infection of all the snails could hardly be expected to occur even for those species of cercariae with the highest incidence; and certainly not in the case of the scarcer forms. For these reasons, it would be expected that the actual number of double infestations found in our collections would fall considerably below the chance expectations even with species of cercariae between which there was no immunity of antagonism. On the other hand, if the frequency of double infestations between certain species approached much more closely to the chance probability than those between others, some conclusions in regard to immunity or antagonism might be drawn. At least, it seemed to us of interest to line up the figures for the actual occurrence of double infestations in our collections against those that would be expected by chance. The results from this procedure have definitely suggested to us that double infestations with certain of the species in our series occur by chance; for others, the small numbers found as compared with the chance expectations suggests an immunity or antagonism. It would be entirely too tedious to present here this analysis for all 17 species of cercariae in all 14 groups. We will, therefore, only present limited examples which illustrate the interesting relations found.

Such an analysis is made in table 3 for all possible combinations in double infestations of the eight commonest species of cercariae for seven selected groups from all four areas. The reader can himself, if he wishes, easily extend this same type of analysis to all the species in any or all the groups presented in tables 1 and 2. From table 3 it can be seen

TABLE 3.—*Analysis of chance occurrence of double infestations for all possible combinations of the eight most common species of cercariae in seven of the groups from tables 1 and 2*

Combinations in double infestations	Burt Lake Area								North Fishtail Bay area		Nutting Bay area		Phragmites Flats area	
	III		IV		V		VI		III		I		II	
	No.	Ch.*	No.	Ch.	No.	Ch.	No.	Ch.	No.	Ch.	No.	Ch.	No.	Ch.
Strigeids + Strigeids														
Cer. *D. flexicaudum* + Cer. *C. communis*	2	2	2	4	2	3	4	3	19	27	21	24	11	12
Cer. *D. flexicaudum* + Cer. *laruei*	0	<1	0	<1	0	3	2	3	5	8	2	4	2	2
Cer. *D. flexicaudum* + Cer. *yogena*	0	<1	0	3	13	15	7	11	7	18	10	17	2	4
Cer. *D. flexicaudum* + Cer. *dohema*	0	<1	0	2	4	10	3	7	0	0	1	2	0	0
Cer. *C. communis* + Cer. *laruei*	0	<1	0	<1	0	<1	0	<1	2	3	0	<1	0	1
Cer. *C. communis* + Cer. *yogena*	0	<1	0	<1	0	1	0	<1	2	6	2	2	1	2
Cer. *C. communis* + Cer. *dohema*	0	<1	0	<1	0	<1	0	<1	0	0	0	<1	0	0
Cer. *laruei* + Cer. *yogena*	0	<1	0	<1	0	1	0	<1	0	2	0	<1	0	<1
Cer. *laruei* + Cer. *dohema*	0	<1	0	<1	0	<1	0	<1	0	0	0	<1	0	0
Cer. *yogena* + Cer. *dohema*	0	<1	0	<1	0	3	1	2	0	0	0	<1	0	0
Strigeids + Schistosomes														
Cer. *D. flexicaudum* + Cer. *stagnicolae*	3	3	28	28	11	25	5	24	42	46	12	16	6	8
Cer. *C. communis* + Cer. *stagnicolae*	2	1	6	7	0	2	2	2	10	15	1	2	3	4
Cer. *laruei* + Cer. *stagnicolae*	0	<1	0	<1	2	2	1	2	1	4	0	<1	1	<1
Cer. *yogena* + Cer. *stagnicolae*	0	<1	0	4	3	8	2	5	2	10	1	1	2	1
Cer. *dohema* + Cer. *stagnicolae*	0	<1	0	3	1	5	1	3	0	0	0	<1	0	0
Strigeids + Plagiorchiids														
Cer. *D. flexicaudum* + Cer. *P. muris*	0	9	0	15	4	40	7	55	0	25	3	36	0	9
Cer. *C. communis* + Cer. *P. muris*	0	4	0	4	0	3	0	4	0	8	0	4	0	5
Cer. *laruei* + Cer. *P. muris*	0	1	0	<1	0	3	0	4	1	2	0	<1	0	<1
Cer. *yogena* + Cer. *P. muris*	0	<1	0	2	4	14	5	13	6	5	1	3	1	3
Cer. *dohema* + Cer. *P. muris*	0	<1	0	1	1	9	1	8	0	0	0	<1	0	0
Cer. *D. flexicaudum* + Cer. *P. proximus*	0	<1	0	0	0	0	0	1	0	14	0	27	0	20
Cer. *C. communis* + Cer. *P. proximus*	0	<1	0	0	0	0	0	<1	0	5	0	3	0	12
Cer. *laruei* + Cer. *P. proximus*	0	<1	0	0	0	0	0	<1	1	1	0	<1	1	2
Cer. *yogena* + Cer. *P. proximus*	0	<1	0	0	0	0	0	<1	1	3	1	2	0	4
Cer. *dohema* + Cer. *P. proximus*	0	<1	0	0	0	0	0	<1	0	0	0	<1	0	0
Schistosomes + Plagiorchiids														
Cer. *stagnicolae* + Cer. *P. muris*	2	6	3	25	17	22	38	29	0	13	3	3	3	3
Cer. *stagnicolae* + Cer. *P. proximus*	0	<1	0	0	0	0	1	<1	6	8	1	2	7	7
Plagiorchiids + Plagiorchiids														
Cer. *P. muris* + Cer. *P. proximus*	0	<1	0	0	0	0	0	1	0	4	0	5	2	9

* Ch. = number expected by chance.

at a glance that there are much greater differences between the actual and the expected number of double infestations for certain species combinations than for others.

One of the best examples of a close approximation between the actual number of double infestations present and the number expected by chance, is in the combination of the cercaria of *Diplostomum flexicaudum* and the cercaria of *Cotylurus communis*. In table 3 the total number of such combinations actually found in the seven groups is 61 as compared with 75 that would have been expected by chance. The fact that the adults of both these species are parasites of gulls, which were constantly present in the areas, would insure extensive exposure of the snails to infection with both these species. When we consider the other combinations of strigeids with strigeids shown in table 3, the correspondence is not quite so close. For example, the cercaria of *D. flexicaudum* is found in double infestations with *Cercaria laruei* 11 times when the total chance expectations were somewhat over 20 times. In combinations of this same species with *C. yogena* and *C. dohema* the relations are 39 as compared with 68 and 8 as compared with 21. When the other seven collections of tables 1 and 2 are analyzed in the same way as those included in table 3, results of the same general type are shown for all the combinations of strigeids with strigeids. When the conditions under which these studies were made are considered, we do not feel that these comparisons give any evidence of any immunity or antagonism between these seven species of strigeids in combining with each other in double infestations.

When we consider the combination in double infestations of the cercaria of *D. flexicaudum* with the schistosome, *C. stagnicolae,* the number of actual combinations is remarkably close to that expected by chance except in two groups (V and VI of the Burt Lake area). This same close correspondence is present also in the seven groups of table 1 not analyzed in table 3. If we exclude groups V and VI of the Burt Lake area from the analysis, we find that in the other 12 groups this combination actually occurred 135 times as compared with a chance expectation of 143 times. This suggests the possibility that some unusual factor was operating in groups V and VI of the Burt Lake area to reduce the number of combinations of these two cercariae found in the collections made in August, 1936. As suggested later this might possibly be a differential death rate of the snails harboring these two cercariae in double infestations. In the actual and chance combinations of *C. stagnicolae* with other strigeid cercariae as shown in table 3, the correspondence is not so close but certainly there is nothing to suggest an immunity or antagonism to the production of such combinations.

When we compare the actual with chance expectations of combinations in double infestations of the strigeids with the two most common

plagiorchiids the relations are entirely different. Between the cercaria of *Diplostomum flexicaudum* and the cercaria of *Plagiorchis muris,* the actual number of double infestations found is very small compared with the large numbers expected by chance because of the high incidence of both these species. If we include the whole 14 groups in this comparison, the actual number of times these two cercariae are found together in double infestations is 16 while the chance expectations are 308. Also there is not a single group from any one of the four areas in which the actual number of double infestations of these two cercariae is at all comparable to the number expected by chance. We are of the opinion, therefore, that there must be an immunity produced by the penetration of the miracidium of one of these species which prevents infection of the same snail by the other; or that there is an antagonism present which prevents the development of the other when one is present. The results of the examination of 288 specimens of *S. emarginata canadensis* from the east shore of Mullet Lake which was made in July, 1936, are of interest in this connection. In this collection there were 236 snails positive for the cercaria of *P. muris* or 81.9 per cent and only 8 per cent of the snails were positive for the cercaria of *D. flexicaudum.* These two cercariae were together in only one snail when the number of double infestations that would be expected by chance was 19. It seems possible here that the unusually low incidence of the cercaria of *D. flexicaudum* in this collection was due to extraordinarily high incidence of the cercaria of *P. muris.*

In relation to the combination of the cercaria of *P. muris* and that of *C. communis,* the immunity or antagonism is just as clear, since this combination does not occur at all in any of our groups in spite of the fact that the incidence of the two species is such that it would be expected to be present by chance in every one of the 14 groups with a total of 45 times. The relations of the cercaria of *P. muris* to the other strigeid cercariae are not so clear. It occurs in double infestations with three of them. For example, in the seven groups listed in table 3 the actual number of times that this species is found in combination with *C. yogena* in double infestations is 17 and the number that could be expected by chance is 39. This is certainly an entirely different relationship from that discussed above for the combinations of the cercaria of *P. muris* with the cercariae of *D. flexicaudum* and *C. communis* and does not suggest an immunity or antagonism.

It will be noted also from tables 2 and 3 that combinations of the cercaria of *P. proximus* with those of *D. flexicaudum* and *C. communis* were never found in double infestations. The incidence of this species is high enough in the eight groups from the North Fishtail Bay, the Nutting Bay, and the Phragmites Flats areas so that these combinations would have been expected 83 and 29 times respectively. Here again, the

evidence seems clear that there must be some sort of an immunity or antagonism which prevents these combinations. Combinations of the cercaria of *P. proximus* with *C. laruei* and *C. yogena* are present and the chance expectations of such combinations are so small that it seems impossible to determine whether they occur by chance or not.

From the above discussion it seems evident that there is some sort of an immunity or antagonism which prevents the occurrence of double infestations of the cercariae of the two most common plagiorchiids and those of *D. flexicaudum* and *C. communis,* but that there is not clear evidence of such a relationship between these cercariae and those of the other strigeids.

The relations of actual to chance combinations of *C. stagnicolae* and the cercaria of *P. muris* are shown in table 3. Although in two groups (IV of the Burt Lake area and III of the Nutting Bay area) the actual number of double infestations falls far short of what would be expected by chance, in all the other groups the correspondence is so close that it seems very probable that this combination occurs by chance. The same relation also appears to hold in the combination of *C. stagnicolae* with the cercaria of *P. proximus.*

The combination of the cercaria of *P. muris* with that of *P. proximus* is present only in two cases (see group II of Phragmites Flats area, table 2). This is the only combination between plagiorchiids that would be expected in this material since the incidence of the other four species is so low. There seems to be some evidence of immunity or antagonism for this combination since in the three areas where it would have been expected to occur at all, the total chances were 28 as compared with its actual presence in only two snails in one group.

As shown in table 2, certain of the other species are not found at all in double infestations. In the case of four of these, the cercaria of *Schistosomatium douthitti,* the cercaria of *Eustomos chelydrae,* Stylet K, and the cercaria of *Notocotylus urbanensis,* it seems probable that this was due to low incidence which made the chances of these species combining with others rather small. For example, for the cercaria of *N. urbanensis* there was only one group of all the collections where according to chance it would have been expected to appear in one case in a double infestation with another species. In most of the groups the same thing can be said also for the cercaria of *Echinostomum revolution.* In certain of the groups, however, the incidence of this species was as high as some of those that were found in double infestations. For example, in group I of the Nutting Bay area it would have been expected to occur in double infestations with the cercaria of *D. flexicaudum* 8 times, and in group I of the North Fishtail Bay area it would have been expected

5 times. This at least suggests that there may be immunity or antagonism that prevented this combination.

As would be expected, occasionally there were instances in the collections of combinations of cercariae appearing in double infestations when their chance of combining was very small. A striking case of this is the combination of Holostome Z with *C. dohema* in group V of the Burt Lake area. The chance of this combination appearing once in this collection is only about 1 in 16. In group V of the Burt Lake area the cercaria of *P. microcanthus* is combined with *C. stagnicolae* in one double infestation with the chances of about 1 in 7, and in group II of the Phragmites Flats area Stylet K and *C. stagnicolae* are found in one double infestation with the chances of about 1 in 15.

From the analysis given above it can be seen that *Cercaria stagnicolae,* as far as the evidence goes, appeared to combine in double infestations with the other species of cercariae in about the numbers that would be expected by chance. Also there is no evidence of immunity or antagonism in the combining of the various species of the stigeids present in these collections with each other. For the two most common plagiorchiid cercariae, however, the evidence seems clear that there was some sort of an immunity or antagonism that prevented combination in double infestations with the two most common of the stigeids, the cercaria of *D. flexicaudum* and the cercaria of *C. communis.* Also these two plagiorchiids do not appear to combine with each other nearly as frequently as could be expected by chance. They do, however, appear to combine with *C. stagnicolae* and with the other stigeids about as frequently as would be expected by chance. While the evidence is, perhaps, insufficient it seems probable that there is some immunity or antagonism between the echinostome and some of the other species of cercariae present since it never appeared in double infestations. The immune or antagonistic relationships appear from the evidence so far to be between certain individual species rather than between whole groups. Finally, there is no evidence from the present work that sheds any light on the mechanism of these immune or antagonistic relationships.

Part II. Differences in Infestations in Different Years and During the Course of the Same Summer

Comparison Between the Collections Made in 1935 and 1936

We have the general impression, based on collections of *S. emarginata angulata* made in the Douglas Lake region over a period of years, that the incidence figures for the various species of larval trematodes that parasitize this snail vary considerably from year to year in the same area. However, the only collections which permit of any very definite comparisons are those of July and August of 1935 and 1936 from three of

the four areas included in our present study (see table 1). It will be seen from this table that in the Nutting Bay area there was a considerably lower percentage of positives in the collections made in August, 1936, as compared with those of the same month in 1935 (cf. groups I and III of this area in table 1). This was due almost entirely to a very striking reduction in the incidence of cercaria of the gull strigeid, *Diplostomum flexicaudum,* which had an incidence in 1936 of less than half that in 1935. The incidence of *C. stagnicolae* is also considerably lower in 1936, but it was also rather low in 1935. For the two common plagiorchiid cercariae the incidence in 1936 is slightly higher than in 1935. The great reduction in the incidence of the cercaria of *D. flexicaudum* might possibly be explained by the fact that in the summer of 1936 there was a larger summer population along this beach and consequently greater activity which would tend to decrease the number of gulls that would come close to the shore.

In the North Fishtail Bay area the only very striking difference between August collections of 1935 and 1936 (cf. groups I and III of this area, table 1) was the very considerable increase in the incidence of the dermatitis-producing schistosome cercariae, *C. stagnicolae,* in 1936. This increase was the chief reason for the larger percentage of multiple infestations found in 1936.

For the Burt Lake area the incidence of infection in the snails was somewhat lower in July and August in 1936 than in 1935 (cf. groups I and II with V and VI for this area in table 1). This difference was produced almost entirely by a reduction in the incidence of the cercariae of *Diplostomum flexicaudum* and *Plagiorchis muris,* since the incidence of the schistosome, *C. stagnicolae,* was considerably greater in 1936 than in 1935, especially in the July collections. Of the other cercariae the only difference which seemed to be of any possible significance was the increase in 1936 of the incidence of *C. yogena* and *C. dohema,* the life cycles of which are unknown.

Taken as a whole the difference between the collections made from these areas in these two years are hardly as great as would be expected. The main features in the collections were much the same in each area for the two years. Of course as would be expected there was considerable fluctuation in the rarer species.

Changes in Infestations During the Course of a Summer

It seemed of interest to determine by collections at different periods during the summer what changes occurred in the larval trematode infestation of *S. emarginata angulata* during the course of a summer. Such collections are available for the summer of 1936 from the four areas that were studied intensively for multiple infestations (see table 1).

Unfortunately on account of the limited period covered by the summer session at the Biological Station these collections only cover a period from June 23 to August 31. It is hoped later to obtain additional information by making collections from these or other areas at earlier and later periods. In the Burt Lake area, the four groups, which include the collections of the summer of 1936 (see groups III, IV, V, and VI from this area in table 1), cover the period from June 23 to August 23. In the other areas there are only two groups each of collections for the summer of 1936 made about seven to nine weeks apart. As we had expected from the records of earlier years, there were very striking changes in the incidence of the different species of cercariae found even over this short period and a very striking increase in the later part of the summer as compared to the earlier period.

One interesting peculiarity in the summer of 1936 needs special mention. An intensely hot wave swept the country in early July which produced the hottest spell for the first 11 days of this month ever recorded at the Biological Station. There were five days during this period when the maximum temperature was over 100° F. The temperature of the water along the beaches where our collections were made very greatly increased during this hot spell. This, of course, must have speeded up the development of all stages of larval trematodes during that period, both the development of the eggs in the water and the development of the immature stages in the snails.

In presenting the material from the collections made at different times during the summer of 1936, the different areas will be first discussed separately, and then the important points will be summarized for the different species of cercariae.

Burt Lake area. In this area four groups (III, IV, V, and VI) of between 400 and 500 snails each are available for comparison. All of these consist of single collections except group VI, which includes two collections made three days apart. As shown in table 1 the first collection (group III) was made on June 23; the second (group IV) on July 11, 18 days later; the third (group V) on August 11, one month after the second; and the two collections of group VI on August 20 and 23, less than two weeks after the third. The snails were rather scarce on this beach so that each collection appeared to be a good sample of those present.

Except for a considerable increase in the incidence of the cercaria of *P. muris* in group VI as compared with group V, the two August groups show very much the same incidence of the various species of larval trematodes. Therefore, the chief interest in this series attaches to the changes that occurred in the seven weeks that separated the first and third collections (groups III and V). In this period the incidence of positives had

almost doubled and the number of multiple infestations had increased from 10 to 64. The changes in incidence of the different species of cercariae can be seen in table 1. Most striking is the increase in the incidence of the cercaria of *D. flexicaudum,* which is about three times as great in the August collections as in the one made in June. Over this period there are no significant changes in the incidence of the cercaria of *C. communis* and *C. laruei,* but both *C. yogena* and *C. dohema* increase greatly. In fact there is only one snail positive for *C. yogena* in the June collection while this cercaria is present in 47 snails in the collection of August 11.

The changes in incidence of *C. stagnicolae* are rather surprising for after an increase from 7.4 per cent to 33.0 per cent in the 18 days from June 23 to July 11 (cf. groups III and IV), the incidence of this species drops in the August groups (V and VI) to 17.8 to 16.5 per cent respectively. If, as seems almost certain, the life history of this species is like that of other schistosomes, it is possible that in as hot a period as early July 1936, the time from the depositing of eggs in the water to a stage that could be identified in the snails might be as short as three weeks. It seems certain, therefore, that the definitive host or hosts of this species must have frequented the beach of the Burt Lake area in large numbers in June to produce the great increase in incidence that developed by July 11. In fact, much of the infestation present at this later date must have been represented in the snails of the collection of June 23 by such early stages that they were not even identified as immature cercariae. Equally striking is the decrease from 33 per cent of *C. stagnicolae* in the July collection to 17.8 and 16.5 per cent in the August collections (cf. group IV with V and VI).

With the cercaria of *P. muris* the changes in incidence in the collections from the Burt Lake area during the summer of 1936 are also of special interest. From a figure of less than 20 per cent in the June and July collections (groups III and IV) the incidence of this species had increased to almost 30 per cent in the month from July 11 to August 11 (group V) and to 38.0 per cent by August 20 and 23 (group VI).

Some interesting suggestions also come out of the changes in certain combinations of double infestations in these collections made from the Burt Lake area during the course of this summer (see table 3). On account of the increase in the incidence of both species, combinations of the cercaria of *D. flexicaudum* and *C. stagnicolae* had increased from 3 to 28 from June 23 to July 11 (cf. groups III and IV), being in both cases exactly the same as would have been expected by chance. In groups V and VI this number had decreased to 11 and 5 respectively which in both cases is very much less than the numbers expected by chance. As noted above these are the only cases in any of our collections

in which the actual number of double infestations of this combination found fail to approximate closely the number expected by chance. It seems possible that this might be explained by a greater mortality in the snails infected by both of these two species. It seems difficult to believe that it occurred by chance.

Except for combinations of *C. stagnicolae* and *C. muris* the changes in multiple infestations in the later collections from the Burt Lake area (see tables 2 and 3) can be explained on the basis of changes in the incidence of the species involved. In this case there were only three double infestations when 25 would have been expected in the collection of July 11 (group IV), while in that of August 11 (group V) there were 17 when 22 would have been expected, and in the collections of August 20 and 23 (group VI) there were 38 when 29 would have been expected by chance. It seems possible that there was some connection between the decrease of double infestations of *C. stagnicolae* and the cercaria of *D. flexicaudum* during this period and the increase of those in which this species was combined with the cercaria of *P. muris*.

It seems possible that infection of snails with *C. stagnicolae* occurred only early in the summer at this beach in 1936. The decrease in the incidence of *C. stagnicolae* by August might then perhaps be explained by a differential death rate of snails infected with this species especially when it was combined in double infestation with the cercaria of *D. flexicaudum*. There was no reduction, however, in the total numbers found in double infestations for the reduction in the number of *C. stagnicolae* that were in combination with the cercaria of *D. flexicaudum* was made up for by the increase in double infestations in which it combined with other species especially with the cercaria of *P. muris*.

North Fishtail Bay area. From this area there are two groups of one collection each for the summer of 1936, one for June 29 and the other seven weeks later for August 17 (see table 1, groups II and III of this area). In the second of these two collections the incidence of positives was more than twice that in the first. The incidence of the four strigeid cercariae present increased greatly. Here as in the Burt Lake area, *C. yogena* showed the most striking increase from 0 to 9.0 per cent. The increase in the incidence of *C. stagnicolae* from 0.2 to 22.9 per cent was perhaps the most striking single thing in these collections. This means that in August dermatitis would have been very easily acquired on this beach if it had been used for swimming. There was also a considerable increase in the incidence for the cercaria of *P. muris*. As would be expected from this great increase of strigeids and the schistosome there was a very great increase in multiple infestation (from 8 to 113) in this area in the August as compared with the June collection (cf. groups II and III of this area in table 2). There was nothing however, in this increase

which can not be explained on the basis of the increase in the incidence of the various cercariae present.

Nutting Bay area. In this area the two groups (II and III) which were almost 10 weeks apart showed few changes of interest since the incidence of positives was not very high even in the collection of the last of August. In fact about the only difference of any significance was an increase in the incidence of the cercaria of *D. flexicaudum* from 11.5 to 17.4 per cent. This increase was the chief reason for the increase of the multiple infestations from 1 to 9.

Phragmites Flats area. In this area the two groups (I and II) consist of two collections made about seven weeks apart. Here the most striking increases are in the incidence figures for the cercaria of *D. flexicaudum, C. stagnicolae,* and the cercariae of the two common plagiorchiids (*P. muris* and *P. proximus*). Here, too, the increase of multiple infestations from 13 to 43 can be explained by the increase in the incidence of the various species.

In considering the data from these four areas from these collections made at the beginning and end of the summer of 1936, some interesting points can be raised in relation to the yearly cycle in this snail of the various species of larval trematodes present. In all of the four areas included in this study, the incidence of the cercaria of *D. flexicaudum* increased greatly during the course of this summer, although in all cases it had a fairly high incidence in the collections made in June or early July. For this species, Van Haitsma (1931, a) found that during the months of August and September the time of development of the eggs up to the hatching of the miracidium was about three weeks and that snails infected September 1st gave off cercariae six weeks later. This makes a total of about nine weeks from the time the eggs are deposited in the water to the completion of development of the cercariae in the snail intermediate host. In the warmest part of the summer this period would, of course, be shorter and in April, May and June it would probably be somewhat longer. To produce the infestations found in our collections in June, eggs of this species would have had to have been deposited in the water by the gulls in April or at latest in early May. Further, it seems clear that part of these early infestations with this species lasted in the snails over the winter since old infestations, as judged by the structure of the daughter sporocysts and the fact that most of the cercariae had already escaped, were found in the earliest collections that were made in the summer. It is also probable that eggs in various stages of development remain viable over the winter, although this has not been demonstrated. Gulls are constantly present on all these beaches during the whole summer. This would account for the great increase in incidence in this species in all the areas during the course of the summer, and sug-

gests that snails were constantly being infected. This is also indicated by the fact that immature infestations that could be identified as those of *D. flexicaudum* were always present in collections, even those made toward the end of August. In the collections made after the last half of August, also, the number of old infestations increased greatly which indicated that some of the infestations which reached maturity earlier in the summer had run their course and were becoming exhausted. It seems very probable, therefore, that snails are infected with the miracidia of *D. flexicaudum* over the whole periods of the year that the temperature is high enough for the hatching of the eggs and the penetration of the miracidia. Those infestations still present in the fall would be carried over the winter without further development and would complete their development and run their course as soon as it was warm enough the next spring. The low incidence figures for the earlier collections indicate either that most of the infestations acquired during the summer ran their course before cold winter set in or that there was a differential death rate during the winter affecting the snails infested with this species. Collections over longer periods of the year will be necessary before it is certain that the picture given above for the yearly cycle of the cercaria of *D. flexicaudum* in *S. emarginata angulata* is the true one.

It is interesting to consider in this connection that the relations in the early and late collections of the cercaria of *C. communis,* which is also a gull form, are quite different from those of the cercaria of *D. flexicaudum,* just discussed. For that species in only one area, North Fishtail Bay, was there a significant increase in the incidence during the course of the summer and in two of the areas there was a considerable decrease. Why there should be this difference in two related species, with the same definitive host and very similar life cycles, is difficult to explain.

For the other strigeids, only the data on *C. yogena* are such as to offer much of interest. In the June collections this species was almost entirely absent and its increase during the course of the summer especially in the Burt Lake and North Fishtail Bay areas was very marked. This would suggest that the definitive host of this species was present on these beaches early in the summer and that the infestation was not carried over the winter. Perhaps infestation with this species occurs only in the first part instead of throughout the whole course of the summer as appears to be the case with the cercaria of *D. flexicaudum.* Records from other collections besides those in this series also show that *C. yogena* seldom appears in collections made in June and early July.

Data on the changes in the incidence of *Cercaria stagnicolae* during the course of the summer are of peculiar interest since this species is the

most important of the dermatitis-producing schistosome cercariae of the region. This species of cercaria appears to account for most of the dermatitis that is so prevalent on certain of the bathing beaches of this general region (Cort, 1936). Our records for the appearance of the dermatitis on the beaches show that it is rare and of very little significance until after the middle of July. This may, of course, be due partly to the fact that the weather is warmer in July and August which attracts more summer resorters to the region, and leads also to a greater use of the bathing beaches. We found, however, from our earlier records that collections of *S. emarginata angulata* made in June and early July rarely showed any appreciable infestation with *C. stagnicolae,* and that it was much more common in the collections of this snail made later in the summer. This relationship was confirmed by the collections of the summer of 1936 which showed striking increases in the incidence of this species during July and August. This is especially clear in the comparison of collections II and III from the North Fishtail Bay area (Table 1). Here on June 29 only one snail was positive for this cercaria while in the collection made on August 17, seven weeks later, there was an incidence of 22.9 per cent. If we assume, as seems almost certain, that the life cycle of *C. stagnicolae* is like that of other schistosomes, the time from the depositing of eggs in the water up to the development of the cercaria in the infected snail to the stage that it can be identified, would be only three to four weeks in the summer. This would mean that in this area the definite host or hosts of this species could not have been depositing eggs in any quantity in the water until almost the middle of June. In the Burt Lake and Phragmites Flats areas the first infections must have been somewhat earlier, since the incidence was 7.4 per cent in the collection from the former area made on June 23 and 3.0 per cent in the later area on July 6. As suggested earlier the very great increase in *C. stagnicolae* in the Burt Lake area between June 23 and July 11 indicates that the snails had a very extensive exposure to infection during June. Whether the reduction in the incidence of *C. stagnicolae* which was found in the Burt Lake area between July 11 and August 11 was peculiar to this particular situation and year or is a general phenomenon we cannot say from our present information. It is of interest, however, that collections I and II from the Burt Lake area made during 1935 suggest that there was a reduction also in that year. All we can say from the present data is that there is evidence that the increase in schistosome dermatitis on the beaches after the middle of July is associated with a greatly increased incidence of *C. stagnicolae.* Also it is apparent in at least two areas that the snails were exposed to infection early in the summer and little if any of the infestation with this species was carried over the winter. Further studies are planned which should give additional data on the yearly cycle of this form.

Of the xiphidiocercariae, the cercaria of *Plagiorchis muris* is the most common. For this species, the records from the Burt Lake area are of particular interest. Here, the incidence of 19.0 per cent in the collection of June 23 suggests very considerable exposure to infection early in the season or a considerable carrying over of the infestation in the snails throughout the winter. The time required for development of this species up to the cercaria stage is not known, but would probably be shorter than for the strigeids. The wide variety of hosts of this form (McMullen, 1937) including very common birds, such as the sandpiper and nighthawk, would account for its high incidence and would give the chance for continued infection throughout the summer. The data on the cercaria of *P. proximus,* a mammal form, with the muskrat as natural host in the region is a little hard to interpret. Only in the Phragmites Flats area was there any appreciable increase in the incidence of this form during the summer season.

Taken together these limited records on the changes in larval trematode infestation in *S. emarginata angulata* over a summer season, indicate that studies of this type can be counted on to give some information on the yearly cycles of infestation of larval trematodes, and perhaps give leads that will be of aid in the clearing up of life cycles.

Summary

Intensive studies of the larval trematodes of the common beach snail, *Stagnicola emarginata angulata,* were made during the summers of 1935 and 1936 from the region of the University of Michigan Biological Station. A total of 7,259 snails of this species were examined from 19 collections from four separate areas, analyzed in 14 different groups. Of these snails, 4,304 snails contained cercariae far enough developed for identification, 255 contained immature infestations that could not be identified, and 2,700 were negative. In all, 17 different species of cercariae were present, part of which were quite common in practically all the areas studied. These belonged to five different trematode groups as follows: strigeids, seven; schistosomes, two; plagiorchiids, six; echinostomes, one, and monostomes, one.

In the positives there were 529 multiple infestations with two or more species of cercariae or 12.3 per cent of the identifiable positives. Of these, 511 were double, 17 triple, and one quadruple. The double infestations occurred in 25 different combinations and the 17 triple infestations were found in nine different combinations. It is suggested that the small number of multiple infestations in the collections of Sewell and Dubois, which led them to postulate the development of some mechanism in the snail host that usually prevented the occurrence of multiple infestations, was due chiefly to the fact that in most of their collections the

incidence of the different species of cercariae present was too low to allow the chance occurrence of any considerable number of such combinations.

A mathematical analysis of our collections to determine whether the actual occurrence of double infestations for the different combinations of species of cercariae agree at all closely with chance expectations, suggested that some combinations occurred by chance and that for others some sort of immunity or antagonism between the two species involved must be present. The common schistosome cercaria, *C. stagnicolae,* appeared to combine with the other cercariae in about the numbers that would be expected by chance under the conditions of the study. Also, there is no evidence of any immunity or antagonism in the combining of the various species of strigeids with each other. On the other hand for combinations of the two most common plagiorchiids, the cercariae of *P. muris* and *P. proximus,* with the two most common strigeids, the cercariae of *D. flexicaudum* and *C. communis,* the actual number of double infestations found fell very far short of the numbers expected by chance. This was interpreted to indicate the presence of some immunity or antagonism that prevented such combinations. Also, these two plagiorchiids did not combine with each other nearly as frequently as would be expected by chance; and the only echinostome present never appeared in double infestations with any other of the cercariae, when in certain cases such combinations would have been expected. What evidence we have of an immunity or antagonism in the formation of double infestations seems to be between individual species rather than between groups.

Collections for certain of the areas were compared for 1935 and 1936. While many differences in the incidence of the different species were found for the two years, they were hardly as great as would have been expected and the main features of larval trematode infestations in the collections were much the same in each area for the two years.

In all four of the areas, collections were made at different times during the course of the summer of 1936. Collections made toward the end of August showed an incidence of positives about twice as large as in those from the same areas made in late June or early July. For certain species, for example, the cercariae of *D. flexicaudum* and that of *P. muris,* the evidence suggested that infection must occur throughout the whole summer and be carried over the winter. For other species, *C. stagnicolae* and *C. yogena,* it appeared that infestations were acquired only early in the summer and were not, to any considerable extent at least, carried over the winter.

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SUMMER MEETING OF THE AMERICAN SOCIETY OF PARASITOLOGISTS

Program arranged under the auspices of the A. A. A. S., Denver, Colorado, June 22, 1937.

TUESDAY MORNING SESSION, JUNE 22, 9:00 A. M.

1. Notes on Some Cimicid Parasites of Man and Animals. George M. List, Colorado State College.
2. Some External Parasites of the Rodent Family Sciuridae. Sam C. McCampbell, Colorado State College.
3. Some Observations on the Biology of Mosquitoes. William B. Owen, University of Wyoming.
4. Notes on Siphonaptera of the Western States. William L. Jellison, Rocky Mountain Spotted Fever Laboratory.
5. Studies on Host Reactions to Larval Parasites. II. Comparison of Two Flesh-Penetrating Cercariae. George W. Hunter, III, Wesleyan University.
6. The Life Histories of Some Gregarines Parasitic in Marine Crustacea. Gordon H. Ball, University of California at Los Angeles.
7. The Geographical Distribution of *Diphyllobothrium latum*. Thomas B. Magath, Mayo Clinic.
8. The Life History of *Diphyllobothrium mansonoides* with some Considerations with Regard to Sparganosis. Justus F. Mueller, Syracuse University.
9. Taxonomic Status of *Diphyllobothrium latum* in Western Canada. Robert A. Wardle, University of Manitoba.
10. The *Diphyllobothrium* Species Found in the Yellowstone Park Region. John W. Scott, University of Wyoming.
11. Sylvatic Plague in Montana. Wm. L. Jellison, R. R. Parker and Gordon E. Davis, U. S. Public Health Service.
12. The Influence of *Diphyllobothrium* Infestation upon Dogs. Robert A. Wardle, University of Manitoba.
13. Carbon Tetrachloride Poisoning in Sheep. J. N. Shaw, Oregon State Agricultural College.
14. Two Species of *Triatoma* (Reduviidae) from Mexico Naturally Infected with *Trypanosoma cruzi* Chagas. B. G. Whitaker, University of California, and L. Mazzotti, Department of Public Health, Mexico.

TUESDAY AFTERNOON SESSION, JUNE 22, 2:00 P. M.

Symposium on Rocky Mountain Spotted Fever

1. A General Survey of the Problem. R. R. Parker, Rocky Mountain Spotted Fever Laboratory.
2. The Rocky Mountain Spotted Fever Tick, *Dermacentor andersoni*. R. A. Cooley, Rocky Mountain Spotted Fever Laboratory.
3. The Virus of Rocky Mountain Spotted Fever in Tissue Culture. Herald R. Cox, Rocky Mountain Spotted Fever Laboratory.
4. Sylvatic Plague. C. R. Eskey, U. S. Public Health Service, Plague Laboratory.

ABSTRACTS

The Life Histories of Some Gregarines Parasitic in Marine Crustacea. G. H. BALL, University of California at Los Angeles.

There is described the complete life-history of a new species of gregarine from the Striped Shore Crab, *Pachygrapsus crassipes* of the Pacific Coast of North America. A comparison is made between this species and the two forms,

Cephaloidophora conformis (Diesing) Leger and Dubocq from *P. marmoratus* of the Mediterranean and *C. communis* Mavrodiadi, parasitic in *Balanus amphitrite* and other barnacles. On the basis of the morphology and the discovery of new stages in the development of the last-named gregarine, the two species from *Pachygrapsus* are placed in a new genus.

The Rocky Mountain Spotted Fever Tick, Dermacentor andersoni. R. A. Cooley, Rocky Mountain Spotted Fever Laboratory.

A brief summary is given of available data on the bionomics and control of *Dermacentor andersoni* Stiles, the carrier of Rocky Mountain spotted fever and other diseases of humans in the western United States. It is the most important tick vector of human disease in this country. The paper includes brief information on the scientific name, distribution, animals on which the ticks feed, life history of the tick and its seasonal history. Information is given on what is popularly called the "tick season," and also includes conditions favoring the presence of this enemy of man, as well as its adaptations and means of dissemination. The fear of ticks is discussed and particular attention is given to the control of ticks in the wild mountainous areas, the valleys and basins within the mountains and the extensive grazing of sage brush areas in the plains. The efforts toward the control of the tick, which have been continued for some twenty-one years and embracing the use of the artificial methods of dipping live stock and killing rodent life are appraised. Particular attention is given to the use of tick parasites for the control of the tick in the mountainous areas, and the only known tick parasites in the world, two in number, were used in these efforts. The author had also spent a year in Africa searching for further parasites to be introduced into this country for the destruction of this tick. In final summary, the author states "that at the present time, we know of no effective method of controlling *D. andersoni* either by artificial means or by parasites and these results serve to give further emphasis to the importance of the control of the Rocky Mountain spotted fever by prophylactic methods."

Sylvatic Plague. C. R. Eskey, U. S. Public Health Service Plague Laboratory.

Sylvatic plague has spread eastward an unknown distance from the Pacific Coast with foci now demonstrated in seven states, and dissemination will continue until lack of a susceptible rodent population bars its progress. Eleven species of ground squirrels are already involved so that these rodents, marmots and prairie dogs appear to be the animals most responsible for the dissemination of plague. Many species of fleas must act as vectors. Preliminary investigations indicate fleas of wild rodents may harbor *Bacillus pestis* for long periods without transmitting plague when biting, although virulent organisms are present in their feces which might infect their hosts. These infected fleas could also bite humans without transmission of infection. Tick borne diseases appear to be more readily transmitted to man than plague. Surveys for sylvatic plague foci are now being conducted by field crews of six infected states and four units from the U. S. Public Health Service Laboratory in San Francisco. Inoculation of fleas collected from wild rodents is proving to be of more value in locating plague foci than the use of tissue from suspicious rodents. Eradicative measures should only be applied according to the conditions present in infected areas as complete control can only be attained by the most intensive, prolonged, and costly procedures.

Studies on Host Reactions to Larval Parasites. II. Comparison of Two Flesh-Penetrating Cercariae. George W. Hunter, III, Wesleyan University.

Two general types of cyst walls are encountered in studying larval helminths. The first type is composed of two layers, an inner hyaline sheath presumably elaborated by the parasite and an outer one from host connective tissue. Examples of this type may be found among many of the Strigeid trematodes of the larval group *Neascus,* as well as in the Heterophyid, *Cryptocotyle lingua.* In the second type there is no inner cyst, but only the outer one of connective tissue derived from

the host. This latter type is represented by *Clinostomum marginatum,* the yellow grub of fish. In the case of the black grub of fish, *Crassiphiala ambloplitis,* the portion of the mature cyst produced by the host is divisible into two parts, an inner mass of dense and outer one of loose connective tissue. The melanophores are confined to this outer part. The relative thickness varies with the host species. In the yellow grub the amount of connective tissue likewise varies with the host, but no melanophores are present.

Sylvatic Plague in Montana. Wm. L. Jellison, R. R. Patker, and Gordon E. Davis, U. S. Public Health Service.

Sylvatic plague was first recognized in Montana in July, 1935, in the study of a widespread epizootic in ground squirrels, *Citellus* spp., in the southwestern part of the state. Three infected squirrels, *Citellus elegans,* were found in Beaverhead County in that year and sixteen in Beaverhead and Madison counties in 1936. The epizootic apparently started in southern Beaverhead County in 1933 and spread northward along the three main river valleys and by 1936 had practically exterminated the heavy ground squirrel populations in the southern halves of Beaverhead and Madison counties. Plague was demonstrated in three well separated areas on the margin of this epizootic but may not have been the only agent involved. The species of ground squirrels present in the area are *Citellus armatus, C. elegans* and *C. richardsoni.* Infection has been demonstrated only in *C. elegans.* These ground squirrels are commonly infested with the following species of fleas: *Neopsylla inopina, W. wenmanni, Opisocrostis labis, O. tuberculatus, Oropsylla idahoensis* and *Thrassis* n. sp.

Notes on Some Cimicid Parasites of Man and Animals. Geo. M. List, Colorado State College.

Five species of Cimicids are known to occur in Colorado. Four of these have been known to bite man. Colorado conditions seem to favor the common bed bug. A reliable fumigating company estimates that 25% of the houses in some cities are infested. Poultry houses are frequently found infested. The bat bed bug, *Cimex pilosellus* Horv. is frequently reported in dwellings. There are authentic cases of it and *Haematosiphon inodorus* Duges. feeding upon man.

The Geographic Distribution of Diphyllobothrium latum. Thomas B. Magath, The Mayo Clinic.

The importance of fish for the food of man throughout the ages has accounted for the wide-spread distribution of *Diphyllobothrium latum.* Its distribution is througout the North Temperate zone and follows the distribution of diaptomi and fishes suitable for acting as intermediate hosts. Records of endemic occurrence of the parasite outside of the North Temperate zone are not reliable. The factors determining the exact location of endemic areas are chiefly the habit of eating raw fish and the introduction of sewage into the lake from which the fish are procured. Evidence is presented which indicates that the worm has been introduced into North America in comparatively recent times, by immigrants, chiefly from the Baltic countries. The evidence that the infection is chiefly spread and maintained by dogs and wild animals is not believed to be sufficient to justify its acceptance.

Some External Parasites of the Rodent Family Sciuridae. Sam C. McCampbell, Colorado State College.

The data given in this paper are from an unpublished thesis completed in 1930 and none of the data has been revised since that date. This fact should be kept in mind since no doubt a number of new records have been made since 1930. There are 28 species and subspecies of Sciuridae that occur in Colorado. A total of 177 individual Sciuridae representing 22 species and subspecies have been examined for external parasites. While only 12 species and subspecies were found to harbor external parasites, there is no evidence to indicate that a single

species is free from the attack of such external parasites as sucking lice, fleas, mites or ticks.

Cololrado records of Anoplura infested Sciuridae may be summarized as:

1. Previous records giving definite hosts	5
2. Previous records giving indefinite hosts	3
3. Records by author giving definite hosts	22
4. Records by author giving indefinite hosts	2
5. New host records by author	8
6. New Anoplura records by author	1

Ticks.—Ticks were collected from 4 host species, and represented 3 tick species.

Mites.—Mites were collected from 3 host species, and represented 2 mite species.

Fleas.—Fleas were collected from 9 host species, and represented 9 flea species.

The Life History of Diphyllobothrium mansonoides *with Some Consideration with Regard to* Sparganosis *in the United States.*—Justus F. Mueller, Syracuse University.

D. mansonoides is of peculiar interest because of its close relationship to *D. mansoni* of the Orient, which causes the human disease, sparganosis, in China and Japan. It has been demonstrated that *D. mansonoides* occurs over a large area in this country, and it is very probable that the cases of sparganosis which have been reported for the United States are traceable to this species. The normal host of the adult worm is the bobcat, *Lynx rufus,* although the domestic cat and dog may also serve as hosts. The first intermediate host is normally one of several species of mice, or the water snake, *Natrix.* The stage which occurs in these last two hosts may also occur occasionally in numerous other animals, and thrives in monkeys, and also presumably in man. Infection of the second intermediate host is by taking in infected Cyclops in drinking water, and this is presumably the method of infection in man in this country. An additional species, even more closely resembling *mansoni* than does *mansonoides,* has been found in Florida where it appears normally to infect the raccoon in the adult stage. It appears that the spargana of these forms occur very commonly in a number of small animals in nature. It is suggested that the genus *Diphyllobothrium* be split up into three genera on the basis of important anatomical characters which seem to be associated with physiological differences.

Some Observations on the Biology of Mosquitoes. William B. Owen, University of Wyoming.

The data presented are taken largely from studies made on the mosquitoes of Minnesota. The distribution of the species known to occur in that State is determined by the major vegetational areas or the factors related to their occurrence. Some data on the significance of association of different species in the larval stage are presented. The mating habits of *Theobaldia inornata* Williston are described.

Carbon Tetrachloride Poisoning in Sheep. J. N. Shaw, Oregon State Agricultural College.

The present report is evidence produced in an effort to reproduce the susceptibility to carbon tetrachloride poisoning in sheep and cattle. Since the susceptibility does not always appear in sheep as it does in cattle and since sheep are more available for experimental work than cattle, sheep were used in most instances. Since others have shown that dogs were made susceptible to this chemical by regulation of diet, such methods were used in sheep. Efforts were made to disturb liver function so as to make it less able to take care of the carbon tetrachloride. In most cases sheep on dry feed were used, sheep that might be considered "folded" sheep. All changes in the diet with the exception of the addition

of alcohol were those that might have occurred under natural conditions. No attempts were made to produce injury to the liver with chemicals unusual to the diet of the sheep. Susceptibility to medicinal doses of carbon tetrachloride could not be produced by any of the methods used. This susceptibility to carbon tetrachloride poisoning in sheep continues to be one of the main problems connected with liver fluke eradication. Without more faith in the remedy for this parasite it is doubtful whether any method of eradication can be successful.

Taxonomic Status of Diphyllobothrium latum *in Western Canada.* Robert A. Wardle, University of Manitoba.

On the basis of accepted criteria for the differentiation of species of *Diphyllobothrium,* diphyllobothriid material from man and dog in the district of Winnipeg, one of the three endemic areas of diphyllobothriosis in North America, is adjudged to be homogeneous and to be specifically identical with authenticated *D. latum* from Russia, Finland and Japan. The classificatory value of various characteristics in Canadian material are discussed.

The Influence of Diphyllobothrium latum *Infestation upon Dogs.* Robert A. Wardle, University of Manitoba.

The influence of infestation with the broad tapeworm, *Diphyllobothrium latum,* upon the serum calcium, plasma phosphorus, haemoglobin content and blood picture of dogs was studied. No significant changes in the calcium and phosphorus levels were observed, the values during the pre-infection and post-infection periods lying between 8.7–10.6 for Ca and 3.8–5.5 for P. The blood picture, from the sixth week onward, was that of a mild anaemia, the red cell count falling from a mean pre-infection value of 4.5 to a post-infection value of 3.39 millions per cubic millimeter, accompanied by occasional poikilocytosis and anisocytosis; no eosinophilia was observed; the haemoglobin content fell from a pre-infection value of 14.28 to a post-infection value of 11.52 grams per 100 ml. of blood. No gastro-intestinal nor neuromuscular disturbances were observed.

Two Species of Triatoma (*Reduviidae*) *from Mexico Naturally Infected with* Trypanosoma cruzi *Chagas.* B. G. Whitaker, University of California, and L. Mazzotti, Department of Public Health, Mexico.

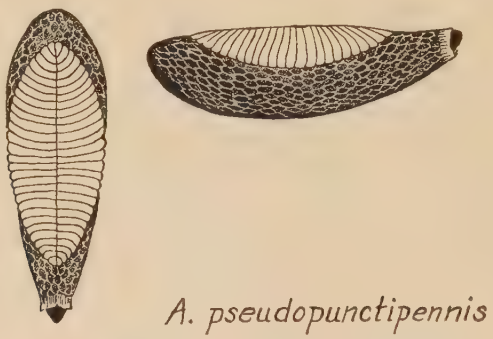
Four out of 24 specimens of *Triatoma pallidipennis* (Stal) Pinto collected in Apatzingán, Michoacon, 2 out of 20 *T. pallidipennis* collected in Tetela, Guerrero, and 104 out of 140 specimens of *T. phyllosoma* (Burm.) Del Ponte collected in Tututepec, Oaxaca, were found naturally infected with *Trypanosoma cruzi.* These two species of bugs may be found in the houses of the natives, especially in the tile and palm-leaf roofs, but are more frequently found in nearby buildings which house domesticated animals or in piles of rocks and lumber near these buildings. They are frequently found in houses far removed from domesticated animals, which would indicate that such bugs subsist on the blood of the human inhabitants and that the frequency with which natives in these areas are bitten by *Triatoma* is greater than the few reported complaints would indicate. Although cases of human infection have not been discovered in Mexico, it is quite likely that they will be found because of the high percentage of naturally infected vectors which occur in close association with the natives. The discovery of a natural infection in these two bugs adds to the already long list of vectors of *Trypanosoma cruzi* and also extends the known range of distribution of naturally infected vectors. The discovery of *T. cruzi* in Mexico lessens the gap in the known distribution which results from a lack of investigation of likely vectors in the area between South America and the United States.

RESEARCH NOTES

THE EGG OF *ANOPHELES PSEUDOPUNCTIPENNIS* IN PANAMA

Investigations in Europe on the puzzling distribution of malaria and mosquitoes have resulted in the discovery of several races of *Anopheles maculipennis*. In the New World, *Anopheles pseudopunctipennis* also has a peculiar relation to malaria transmission; in some regions it is of no importance, while in others it habitually frequents houses, attacking man viciously, and is a dangerous vector. Variations in egg structure do not always signify the presence of more than one race of mosquitoes, but since the habits of *A. pseudopunctipennis* suggest that it may be divided into geographical races, it might be of interest to compare the eggs from different regions. For this reason a description of the eggs from Panama is presented.

The eggs were collected from mats of green algae growing in a creek near Pedro Miguel, Canal Zone.



Description of the Egg (Fig. 1)

FIG. 1. Egg of Anopheles pseudopunctipennis

Length: There was considerable variation in the length, the extremes being 480 microns and 575 microns. Of 217 measurements, the mean was 520 microns and the standard deviation 19 microns.

Float length: Extremes, 320 microns to 427 microns. Of 188 measurements, the mean was 376 microns and the standard deviation 23 microns.

Width: Extremes, 130 microns to 178 microns. Of 99 measurements, the mean was 158 microns, the standard deviation 11 microns.

Long, narrow eggs; the anterior end blunt, and the posterior end tapering. Dorsal surface flattened, ventral surface convex. Color black. The exochorion possesses the pattern of polygonal markings that has been described for eggs of other members of the Anopheles Group, and covers all of the egg except for the posterior tip, where the exochorion flares out into a broad, collar-like frill. The floats are large; they are placed on the dorsal surface, and are fused with one another along the mid-dorsal line. There is a wide range in the number of float ridges, the extremes being 22 and 38, although most of the floats have from 26 to 33 ridges. Of 307 float ridge counts, the mean was 29.6, and the standard deviation 2.7.

A. pseudopunctipennis eggs of Panama resemble the eggs of this species that were described from California by Herms and Freeborn (1920, Jour. Parasit., 7: 69–79) except that in the Panamanian form the floats are large and have many float ridges, while the egg described by Herms and Freeborn the floats are smaller and are "represented by a fusiform closely appressed area," and there are only "twelve sections representing the twelve original compartments of lateral floats."

The collar-like frill of the local eggs is identical with that described by Herms and Freeborn. Herms and Frost (1932, Jour. Parasit., 18: 240–244) described a different kind of *A. pseudopunctipennis* egg from California, and suggested that an inward fold of the floats might have been mistaken for the "collar-like float" (frill?) by Herms and Freeborn. In the eggs from Panama there is no possibility of confusing the frill and floats; both are distinct and separate, and except for size and number of ridges, the structures of the floats shows no variation. It would be interesting to know if the various types of California eggs can be deposited by the same mosquito, or if they represent seasonal variations such as those described for *A. walkeri* by Matheson and Hurlbut (1937, Amer. Jour. Trop. Med., 17: 237–243).—Lloyd E. Rozeboom, *Medical Entomologist, Gorgas Memorial Laboratory, Panama, R. de P.*

FACTORS INFLUENCING THE DEVELOPMENT AND VIABILITY OF THE EGGS OF *NIPPOSTRONGYLUS MURIS*

In his study on *Nippostrongylus muris* development, Yokogawa (1922, Parasit., 14: 127–166) noted that the majority of the eggs in liquid cultures hatched in 24 hours and the emerging rhabditiform larvae became filariform in four to five days. While he did not critically study the effects of temperature on the rate of development, he noted that it was more rapid during the summer period than during the spring and autumn. The same worker found that the eggs would not develop in water cultures over 2 mm. deep probably because of a reduced oxygen supply. In addition, the eggs did not complete their development in cultures that were not saturated with moisture. Africa (1931, Jour. Parasit., 17: 146–206) reported that a 24-hour exposure to freezing temperatures was lethal to most of the eggs.

It is the purpose of the present paper to give the findings on the effect of various temperatures and decreased moisture upon the viability and rate of development of *N. muris* eggs. The strain used was isolated from a wild rat in Baltimore and maintained in laboratory rats during these experiments. The preliminary experiments made with pellet cultures supported the work of Yokogawa and Africa cited above. However, it seemed desirable to have more quantitative information so the following technique was employed. The eggs were isolated from the feces of infected animals by saturated salt flotation and washed and concentrated as described by Hunninen (1935, Jour. Parasit., 21: 124–125). The salt flotation is usually carried out in a few minutes and since it was found that the eggs of *N. muris* were not affected by flotation with a concentrated sodium chloride solution up to an hour's time the procedure was not detrimental to the eggs. The influence of the various temperatures was tested by placing a portion of the washed eggs into water 2 mm. deep in Syracuse dishes and exposing these cultures to test temperatures for periods from 3 to 52 hours as indicated in table 1. Eggs exposed to temperatures other than 22 and 30° C. were returned,

TABLE 1.—*The percentage of eggs of* Nippostrongylus muris *in water cultures that survived exposures to the listed temperatures and later hatched at room temperatures (21–24° C.)*

Temperature	Hours exposed	Percent of hatched eggs
−5° C.	3	17
	6	5
	10, 12, 24	0
0° C.	10	80
	24	56
	48	38
	52	22
22° C.	24	95
30° C.	24	95
35° C.	6	85
	10, 12, 24, 48	0

after the test interval indicated, to water at room temperatures (21–24° C.). The viability of these cultures was tested by examining samples of at least 100 eggs from each culture for four to five days and recording the percentage that hatched as the index of viability.

The results with the water cultures of the eggs of *N. muris* are embodied in table 1. It is noted that none of these eggs were alive after 10 hours exposure to −5° C. However, if the temperature of exposure is increased 5 degrees to 0° C., over 20 per cent of the eggs were still viable after 52 hours of such exposure. Confirming Yokogawa again, it was found that most of the eggs in the fluid cultures at 22 and 30° C. were hatched by the end of the first 24 hours. Because of the high rate of development in temperatures from 22–30° C. the cultures in this range, which no doubt includes the optimum temperature for development, were used as controls. In contrast, all the eggs were killed by 10 or more hours exposure to 35° C. This temperature, which is almost that of the body, proved just as lethal to the eggs of *N. muris* as did −5° C.

The influence of reduced humidities was tested by placing a drop of concentrated egg-material on each coverslip and by allowing the coverslip cultures to dry in room conditions. These cultures were then placed in various test atmospheres. Some of the humidities were experimentally produced and used in the fashion described by Otto (1929, Amer. Jour. Hyg., 10: 521–525). The eggs treated in this manner in relative humidities of 42, 40–55, and 75–80 per cent at room temperatures, 21–25° C., failed to develop and hatch. In fact, only a five-minutes exposure to a relative humidity of 42 at 25° C. killed 95 per cent and 10 minutes killed all of them as evidenced by a 5 and 0 per cent of hatching when returned to water after these exposures.

The qualitative results with the pellet cultures indicated the same general range of resistance of the eggs of *N. muris* to different temperatures and humidities as in the series of water cultures.—George Luttermoser, *Department of Helminthology, School of Hygiene and Public Health, The Johns Hopkins University.*

A FURTHER NOTE ON *EIMERIA MUSTELAE* AND *EIMERIA VISON*, COCCIDIA OF THE WEASEL AND MINK

Yakimoff published in the Journal of Parasitology (1937, 23: 213) a Research Note entitled "A Question of Priority in the Use of the Name *Eimeria mustelae*." Correctly, the author clalims, Iwanoff-Gobzem has priority in the use of the name *Eimeria mustelae* for the Coccidium of the weasel (*Mustela nivalis*), indigenous to Kasakhstan, Middle Asia (Iwanoff-Gobzem, Deutsche Tierärzt. Wochenschrift, 1934, Mar.: 149–151). Pending publication, at the time the article to which reference has just been made appeared, the same name, *Eimeria mustelae*, was proposed for an entirely dissimilar parasite of the North American mink (*Mustela vison*), (Kingscote, Jour. Parasit., 1934, 20: 252–253). Yakimoff, in conclusion to his note, suggests that another name should be chosen for the Coccidium found in the mink.

In 1934 the existence of Iwanoff-Gobzem's right to priority was recognized and the new name, *Eimeria vison*, was given to the parasite of the North American mink (*Mustela vison*), (Kingscote, Jour. Parasit., 1935, 21: 126).—A. A. Kingscote, *Ontario Veterinary College, Guelph, Canada.*

HOST SPECIFICITY OF THE MYXOSPORIDIA OF *FUNDULUS HETEROCLITUS* (LINN).

Regarding host specificity among the Myxosporidia Davis (1917: 214) states, "As a general rule, each species of Myxosporidia is confined to one, or at most a few, closely related species of fishes, but to this rule there are numerous exceptions, and in some cases the parasites may occur in very different hosts, as, for example, *Sphaeromyxa balbianii*, which occurs in the gall bladder of *Motella maculata* and *Siphostoma floridae*." A number of the Myxosporidia have a

great number of hosts accredited to them. Such species as *Myxidium incurvatum* Thel. with 13 hosts, *Chloromyxum leydigi* Ming. with 20 hosts and many others with from 6 or 7 to 10 hosts.

An attempt was made to find other species of hosts for the species of Myxosporidia that occur in *Fundulus heteroclitus*. Fishes of various species were examined. Since the larger fishes of the same area use such forms as *F. heteroclitus* as a major article of food, they must be frequently exposed to great numbers of parasites. If no specific host relationships existed one would expect to find infection in at least some of the fishes of the same habitat. Specimens of all species of fish known to inhabit the areas, that is those areas from which the *F. heteroclitus* were obtained, were collected and dissected in search of any myxosporidian infection. Several species were brought into the laboratory and together with various Poecilidae were fed infected tissues over periods of from two to several months.

From the Chesapeake Bay at Baltimore four young carp (*Cyprinus carpio*), four pickerel (*Esox niger*), eighteen white perch (*Morone americana*), seven yellow perch (*Perca flavescens*), fifteen young menhaden (*Brevoortia tyranus*), twelve *Notropis hudsonius* and seven silver sides (*Menidia* sp.) all proved negative for Myxosporidia. These fish were species commonly found in the same locality or that were usually caught in the same seine hauls with the *F. heteroclitus* of the locality. Though the *F. heteroclitus* of the locality were infected with *Myxosoma funduli* Kudo, *M. subtecalis* Bond, *Myxobolus bilineatum* Bond, *Myxidium folium* Bond, *Sphaerosphora renalis* Bond, and two species of Microsporidia (Bond, Trans. Am. Micro. Soc., in press), very few Myxosporidia were found. Three of eight eels (*Anguilla bostoniensis*) contained Myxosporidian cysts on their gills that proved to belong to three different species of *Myxidium*.

From the Middle River, 15 miles south of Baltimore, the following were collected and examined. Twelve yellow perch (*Perca flavescens*), were found negative; four pickerel (*Esox niger*), one of which was infected with a species of *Henneguya*; five eels (*Anguilla bostoniensis*), two of which carried cysts of the previously found *Myxidium* spp. and one of which was infected with an additional species of *Myxidium*; and six common sunfish (*Eupomotis gibbosus*), one of which was infected with a species of *Cheromyxum* in the gall bladder. Many *Fundulus diaphanus* were dissected and one individual was found to carry an infection of *Myxosoma funduli*. The *F. heteroclitus* of this area were infected with *Myxosoma funduli, M. subtecalis, Myxobolus bilineatum,* and *Myxidium folium*.

Feeding experiments were set up to test infection of various species of fish. The fish were kept in five gallon aquaria and fed infected tissues from *Fundulus heteroclitus* with the addition of chopped liver to make up a sufficient diet. At the end of each experimental period the fish were killed and examined by smear and section.

One eel (*Anguilla bostoniensis*), three yellow perch (*Perca flavescens*), and four sun fish (*Eupomotis gibbosus*) were obtained and fed tissues of *Fundulus heteroclitus* containing an abundance of spores of *Myxosoma funduli, M. subtecalis, Myxidium folium* and *Myxobolus bilineatum*. The eel was fed parasites from December 16, 1936 to March 30, 1937; the perch were exposed to infection from March 10, 1936 to May 20, 1936; and the sun fish were fed tissues from November 20, 1936 to April 3, 1937. Subsequent dissections indicated that all fish were negative for myxosporidian forms. In addition to the above, young *Xiphophorus helleri* were fed spores of *Myxosoma subtecalis* and *Myxobolus bilineatum* from March 14, 1936 to May 29, 1936. Four mature *X. helleri* and four *Platypoecillus maculatus* were exposed to *Myxosoma subtecalis* and *M. funduli* during 4 and 3 successive days, respectively. At the end of sixty-two days the fish were dissected. Five *Lebistes reticulatus* were also studied two and one-half months after exposure to infection for three days. The results of all attempts to infect the above fish with Myxosporidia from *F. heteroclitus* were negative.

In another series of experimental infections using *Fundulus diaphanus* as

the host it was found that this species is susceptible to infection with *Myxosoma funduli, Myxosoma subtecalis* and *Sphaerosphora renalis* from *F. heteroclitus.* Previous studies (Bond, ibid.) reported *Myxosoma funduli* from *F. heteroclitus, F. diaphanus,* and *F. majalis,* and also *Myxosoma subtecalis* has been lately found in *F. diaphanus* of the Hudson River. It is concluded that the Myxosporida of *F. heteroclitus* are confined in their host selection to species of the genus *Fundulus.* The other fishes of the same habitat, although frequently exposed to an abundance of spores, since they feed on *F. heteroclitus* are apparently not susceptible to these parasites.—Franklyn F. Bond, *Department of Protozoology, School of Hygiene and Public Health, Johns Hopkins University.*

A PROBABLE CONSTITUENT OF THE SPORE COAT OF MYXOSPORIDIAN SPORES

The nature of the spore coat of myxosporidian spores has been studied by various authors. Kudo (1921 : 62) summarized the general knowledge by stating, "The spore membrane of Myxosporidia does not give a positive cellulose reaction, and seems to be of chitinoid nature." The spore coat is laid down while the spore is still within the pansporoblastic membrane. The formation is a gradual process occurring through the later stages of spore development, i.e., during the period when the spore structures are being oriented in their final position. This process of spore-coat formation and also the hardening and shrinking of the coat probably continues until the spore is mature. Evidence of prolonged development of the spore coat has been noted in another publication (Bond, Trans. Am. Micro. Soc., in press) under the description of the spores of *Myxidium folium* Bond. Here two types of spores were found, striated and non-striated, according to the age of the spore.

Studies were carried out following observations on material of *Myxosoma funduli* Kudo stained with Mallory's triple connective tissue stain and also Feulgen's nuclear reaction. It was observed that in the same cysts spores would be colored either bright red or golden yellow by Mallory's stain. Spores that stained red were still within the pansporoblastic envelope or showed evidence of being immature, e.g., with six nuclei. The yellow-stained spores were noticeably more mature and in most cases free from the pansporoblastic envelope. The more mature spores with the Feulgen stain became a bluish tinge, the immature spores remaining unstained. Since the staining quality of the spore coat appeared to be associated with the age or maturity of the spore, these studies were instituted in an attempt to establish whether or not there is a relationship between the maturity of the spore coat and its staining reactions.

Both sections and smears of various spores were treated with Feulgen nuclear reagent and counterstained with Orange 'G', and with Mallory's triple connective tissue stain. Observations were made on *Myxidium folium* Bond, *Myxosoma subtecalis* Bond, and *Sphaerosphora renalis* Bond. All of the above species were obtained from *Fundulus heteroclitus* (Linn.) (Bond, ibid.) with the exception of *Myxosoma grandis* material obtained from *Notropis hudsonius* (Girard).

The presence of spores stained either red or yellow and either bluish or unstained was revealed in all of the species when treated by these two methods. The spores colored red with Mallory's stain were found mainly within the pansporoblastic membrane, though a few were found free. The spores that were golden yellow in color were free and mature or nearly mature. The same relationships were found in spores colored by the Feulgen method. The spore coat in certain spores was noticeably colored by this stain. The details of structure were very clear in these preparations; the spores that became stained were mature or were spores in which the valve nuclei had degenerated and the capsular nuclei were pyknotic. The stain was most intense in those spores in which both the valvular nuclei and the capsular nuclei had disintegrated. Young spores and those within pansporoblastic envelopes did not take the stain and the spore coat was untinged with the red-violet of the Feulgen reaction.

The Feulgen reaction is normally dependent on the presence of thymonucleic acids for its appearance, hence the presence of this stain in the spore coat in mature spores indicates that some such substance is present in the spore coat. Salivary digestion for one hour at 37° C. does not affect this reaction, eliminating a possibility of the presence of a polysaccharide. The coloration of the spore coat is correlated with the maturity of the spore, which involves the degeneration of valvular and capsular nuclei. This suggests that the products resulting from the degeneration of nuclei are incorporated into the spore coat and the resulting change in staining reactions is due to the presence of these products.—FRANKLIN F. BOND, *Department of Protozoology, School of Hygiene and Public Health, Johns Hopkins University.*

NEW ANOPHELINE AND CULICINE MOSQUITOES FROM SIAM

In a recent paper the author (1937, Amer. Jour. Hyg., 25: 400–420) showed that while the mosquito fauna of Siam is quite different in the aggregate from that of any of the neighboring countries, most of the species found have been previously described from Malaya, India, French Indochina, or some of the Pacific islands. In the analysis of a large collection, however, several new species were discovered. Two of these are the subject of this communication. The first, an anopheline, is rather unusual because of the concentration of scales on the tarsi of the 2nd and 3rd pair of legs giving them a tufted appearance.

Anopheles bulkleyi new species. Male. *Head:* proboscis, palpi, and antennae all dark without white markings; occiput mainly dark with few white scales along median line. *Thorax:* integument dark and ash gray with brownish collar; prothoracic lobes covered with brown scales; mesonotum sparsely clothed with small whitish hairs; pleurae mainly ash gray, scales wanting, but some evidently rubbed off. *Legs:* heavily clothed with brownish scales except for few white scales on coxae; tarsi of second pair of legs with dark scales in collar-like cluster on second tarsal segment, and on third pair of legs larger cluster on distal end of first and all of second tarsus. *Abdomen:* clothed with luxuriant growth of long brown hair; scales absent except on terminal segments where they form large brown cluster over dorsal, ventral, and lateral portion of hypopygium. *Wings:* with widely-spaced black and white scales; costa with faint white spot at tip of subcosta and first longitudinal vein; proximal half of subcosta denuded of scales, but apical half all dark; first vein with three small white spots on distal half and four on basal half, the largest one situated just basal to origin of third vein; second vein all dark except for scattered white scales on first branch and patch of white scales on second branch; third vein mainly white with few dark scales on basal half; fourth vein mainly dark except for few white scales on first branch and large central white spot on second branch; fifth vein with two dark spots on stem, three on first branch and one on second; sixth vein all white except one dark spot beyond mid point.

This species is described from one male which was bred from larva found in a tree-hole near Chandburi, Siam, and is named for L. C. Bulkley, Trang, Siam. The type male has been deposited in the U. S. National Museum.

Aëdes (*Finlaya*) *hegneri* new species. Male. *Head:* proboscis and palpi of about equal length; proboscis dark dorsally except for small white ring beyond middle and few white scales on sides just posterior to labellae, ringed ventrally by four dark bands one at each end and two in middle third separated by white band of equal width; palpi dark with white rings at base of third and terminal segments, hairs long on 5th, 4th, and apical half of 3rd segment; occiput with large median patch of white scales extending to eye-margin, broad stripe of white scales bordering eye-margins and posterior dorsal portion of head. *Thorax:* prothoracic lobes with large patch of white scales; mesonotum dark brown with yellowish white scales forming distinct lines as follows: double median lines extending to bare space in front of scutellum, another line on each side of these extending whole length of mesonotum, a third pair of lines along the outer mar-

gin curving abruptly inward about middle of mesonotum and paralleling other lines to scutellum; pleurae with several patches of white scales; scutellum with large patch of white scales covering portion of all lobes. *Legs:* coxae with patches of white scales; femur with line of white scales on anterior and posterior aspects; first pair of legs, tibia with white line on posterior and incomplete line on anterior

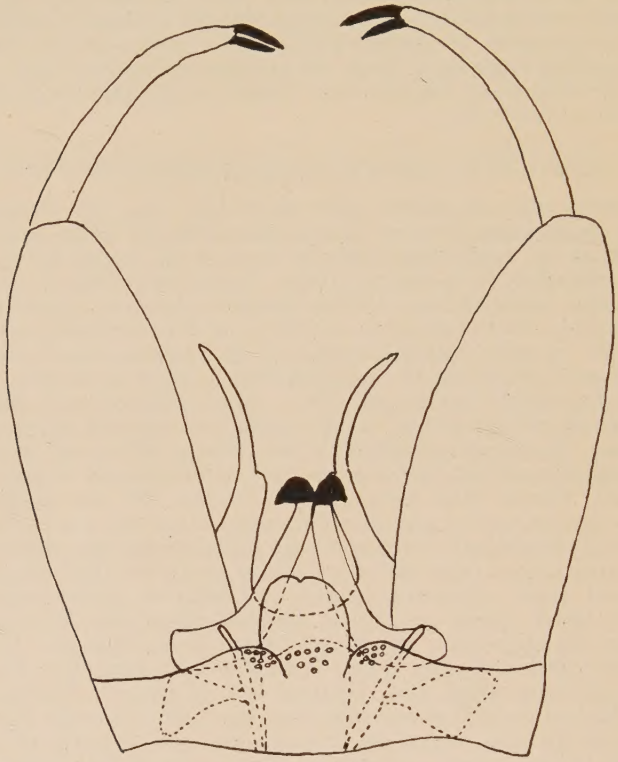


FIG. 1. Terminalia of *Aëdes* (*Finlaya*) *hegneri* new species.

aspect, tibia-tarsal joint with narrow white ring, tarsi with few white scales on joints of 2nd and 3rd segments; second pair of legs, tibia with line of white scales, tarsi with pale scales at base and apex of first and base of second segment; third pair of legs, tibia with white scales on anterior distal quarter, tarsi with white ring at base and distal end of all segments except fifth which is all white. *Abdomen:* dark with basal white rings broader dorsally than ventrally; large patch of white and yellowish scales in center of each tergum of 1st, 2nd, 3rd, 4th, and 5th segments, smaller patches on 6th, 7th, and 8th segments; in some specimens patches divided into two spots by median dark line. *Hypopygium:* ninth tergite bilobed with nine or ten long hairs on each lobe; ninth sternite bearing median patch of nine or ten long hairs; harpago and tenth sternite similar to those of *Aëdes macfarlandi* (see figure 1); clasper with two or three terminal spines.

Female. Not different from male except in head markings; proboscis dark dorsally except for white ring about two-thirds distance from base, and few white scales just posterior to labellae, basal two thirds of ventral aspect white except for narrow basal black area, apical third dark; pattern of occiput similar to that of male but with greater preponderance of white scales; palpi short, about one-quarter length of proboscis, black except at tip which is covered with silver white scales.

This species is described from eight males and four females that were bred from larvae found in rock pools at Chang, Siam, and is named for Dr. Robert Hegner, Johns Hopkins University, School of Hygiene and Public Health. Type specimens have been deposited in the U. S. National Museum.—O. R. CAUSEY, *Department of Protozoology and Medical Entomology, School of Hygiene and Public Health, Johns Hopkins University.*

PRECOCIOUS DEVELOPMENT OF THE METACERCARIA STAGE OF *DIPLOSTOMUM FLEXICAUDUM* IN THE SNAIL INTERMEDIATE HOST

Over a period of several years, diplostomula have been occasionally found in individuals of the common beach snail, *Stagnicola emarginata angulata* (Sowerby) at the University of Michigan Biological Station. The fact that they were found only in snails infected with the cercariae of *Diplostomum flexicaudum* (Cort and Brooks) suggested the hypothesis that they were the precociously developed metacercariae of this species.

During the last three summers, extensive studies have been made on the larval trematodes in this species of snail, involving the microscopical examination, after autopsy, of about 10,000 individuals. Some snails containing the diplostomula were almost always present in collections containing any numbers of the cercariae of *D. flexicaudum*. For example, in a series of collections totaling 1827 snails made in August, 1935, 58 of the 669 positives for this cercaria harbored them. In some cases, only a few would be found among the cercariae in specimens in which the livers had been teased apart. In others, various stages of development were present; and in a few snails large numbers were present both freed in the water and packed in distended sporocysts. It is thought that in the living snails these diplostomula are always within the sporocysts, but this could not be determined with certainty under the conditions of the examinations. They were most common in the snails examined in early June and late August. Their presence seemed to be associated with old infections of the cercariae of *D. flexicaudum*; since they were found with old, often empty, sporocysts. In many cases, they were found with infections in which many of the cercariae were abnormal, having their surfaces covered with knobby protuberances and their bodies and tails vacuolated and swollen. In a few cases, they were found in snails which contained only a very few cercariae and empty sporocysts. There was, however, a considerable percentage of old infections in which these diplostomula were not found.

From our studies, it seems certain that these diplostomula in *S. emarginata angulata* represent a precocious development of the metacercaria stage of *D. flexicaudum* in the intermediate host. In the first place, they have never been found except in snails infected with the cercariae of this species. Further, they agree in structure with the description by Hughes and Berkhout (1929, Pap. Mich. Acad. Sci., Arts and Let., 10: 483–488) of the diplostomula from the lens of the eye of the common sucker, which Van Haitsma (1931, Pap. Mich. Acad. Sci., Arts and Let., 13: 483–516) showed experimentally to be the metacercaria of *D. flexicaudum*. Finally, their development from the cercariae of this species was clearly established by the occasional finding of all the developmental stages including the fully formed diplostomulum with the cercarial tail still attached.

These diplostomula of *D. flexicaudum,* which had developed in the snail intermediate host, were for the most part considerably smaller than those described by Hughes and Berkhout (l. c.) from the normal habitat, the lens of the eye of the sucker. The measurements of these authors gave the following averages for a series of ten specimens: length, 0.417 mm. (range, 0.375–0.465 mm.); width, 0.302 mm. (range, 0.270–0.375 mm.). Three series of measurements of 50 diplostomula each selected at random from three different snail hosts and killed in hot 5% formalin gave the following average dimensions: (1) length, 0.295 ± 0.0022 mm. (range, 0.252–0.350 mm.); width, 0.178 ± 0.0011 mm. (range, 0.154–0.203 mm.);

(2) length, 0.372 ± 0.0019 mm. (range, 0.336–0.434 mm.); width, 0.144 ± 0.0009 mm. (range, 0.126–0.168 mm.); (3) length, 0.378 ± 0.0061 mm. (range, 0.196–0.490 mm.); width, 0.171 ± 0.0017 mm. (range, 0.126–0.210 mm.). In these measurements, the range of variation in each series and the differences between the series are quite striking. This suggests that we are dealing with series which contain numbers of individuals that had not developed to full size. The largest of the individuals in these series approached, especially in length, the sizes given by Hughes and Berkhout. A series of 10 of the best developed individuals from series 3 had an average length of 0.420 mm. and a width of 0.178 mm. It also seems possible that the conditions in the snail host somewhat retard the development of the diplostomula of *D. flexicaudum*.

It is interesting in this connection to mention *Diplostomulum browni* described by Hughes (1929, Occ. Pap. Mus. Zool. Univ. Mich., no. 202, pp. 1–28) from a single specimen of *S. emarginata angulata* in the Douglas Lake region. This form agrees in structure with the metacercaria of *D. flexicaudum* except in its smaller size and slighter development of the lateral suckers. It was in a snail infected with a strigeid cercaria, which the authors did not determine. It seems very probable that these diplostomula were precocious metacercariae of *D. flexicaudum* which had not attained their full size.

As far as we are aware, this is the first case reported of precocious metacercarial development of a strigeid species in its intermediate host. Such development has been reported for *Plagiorchis muris* by Tanabe (1922, Okayoma Igakki Zasshi, 47–68) and McMullen (1937, Jour. Parasit., 23: 235–243) and for *P. proximus* by McMullen (l. c.). For the first of these species, adults were obtained in experimental hosts by feeding the metacercariae that had developed precociously in the snail intermediate host. In the case of *D. flexicaudum*, no attempt was made to infect the definitive host, the gull, with the precociously developed metacercariae.—W. W. Cort and Sterling Brackett, *University of Michigan Biological Station and Department of Helminthology, School of Hygiene and Public Health, The Johns Hopkins University.*